(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
8 December 2005 (08.12.2005)

PCT

(10) International Publication Number
WO 2005/116076 A2

(51) International Patent Classification[7]: C07K 16/30

(21) International Application Number:
PCT/IB2005/002480

(22) International Filing Date: 26 January 2005 (26.01.2005)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
10/764,730 26 January 2004 (26.01.2004) US

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 2005/116076 A2

(54) Title: NEOPLASM-SPECIFIC POLYPEPTIDES AND THEIR USES

(57) Abstract: The present invention features novel polypeptides and methods of using these polypeptides in the diagnosis, detection, monitoring, and treatment of neaplasms in mammal, e.g., a human.

NEOPLASM-SPECIFIC POLYPEPTIDES AND THEIR USES

Background of the Invention

The present invention is related to the field of cancer diagnosis and treatment and, more specifically, to polypeptides and methods of using these polypeptides in the diagnosis, detection, monitoring, and treatment of neoplasms in a mammal, e.g., a human.

In the United States well over one million individuals are diagnosed with cancer each year. Although recent advances in the medical field have significantly improved the rate of survival among cancer patients, a large number of cancer-related deaths still could be prevented by the early diagnosis of the tumor. Accordingly, at the time of initial diagnosis, an alarming number of patients have already reached late stages of the disease. Clearly, there is a need for the early and improved detection and treatment of neoplasms (e.g., stomach adenocarcinoma, colorectal adenocarcinoma, lung adenocarcinoma, adenocarcinoma of the pancreas), as this would increase the chance of treating the neoplasm and, thereby, lead to an improved prognosis for long-term survival.

Human monoclonal antibodies produced from B-cell hybridomas may be used in the treatment of tumors, viral and microbial infections, B-cell immunodeficiencies with reduced antibody production, and other impairments of the immune system. Gastric carcinoma is one of the most frequently occurring types of cancer worldwide and is histologically divided into diffuse adenocarcinoma and intestinal adenocarcinoma. Intestinal gastric carcinomas are often accompanied by chronic type B gastritis and particularly by intestinal metaplasias, which are considered to be precursors of dysplastic changes and of gastric carcinomas. Differences between these two types of gastric carcinomas are also evident in that patients having carcinomas of the diffuse type often belong to blood group A, from which the influence of genetic factors on the cancer risk may be concluded, while environmental factors, e.g., a *Helicobacter pylori* infection, is possibly significant for the occurrence of carcinomas of the intestinal type.

The development of stomach cancer is a multi-step and multi-factor process (Correa, Cancer Res. 52:6735-6740 (1992)). Although little is known about molecular mechanisms, factors such as high salt intake, alcohol, nitrosamines, and infection with the bacterium *Helicobacter pylori* (*H. pylori*) are clearly proven to be involved in the initiation of stomach carcinogenesis. Due to a strong correlation between *H. pylori* infection and the occurrence of gastritis, dysplasia, and development of gastric cancer, the bacterium has been classified as a class I carcinogen by the WHO. *H. pylori* directly induces serious precancerous cellular changes in the mucosal environment and is also responsible for the increase of autoantibodies, which are frequently observed in gastritis and stomach cancer patients (Negrini *et al.*, Gastroenterol. 111:655-665 (1996)). These antibodies are able to induce gastric lesions and apoptosis in the gastric epithelium (Steiniger *et al.*, Virchows Arch. 433:13-18 (1998)). For example, antibodies against the gastric H+/K(+)-ATPase (Claeys *et al.*, Gastroenterology 115:340-347 (1998)), Interleukin-8 (Crabtree *et al.*, Scand. J. Immunol. 37:65-70 (1993); Ma *et al.*, Scand. J. Gastroenterol. 29:961-965 (1994)) and Lewis blood group antigens (Appelmelk *et al.*, Trends. Microbiol. 5:70-73 (1997)) are frequently found in stomach mucosa or stomach cancer.

Therapeutic methods for treating stomach cancer are currently restricted to gastrectomy and lymphadenectomy. Due to the poor prognosis associated with these methods, there is a need for alternative or additional therapeutic and diagnostic methods.

Summary of the Invention

The present invention features polypeptides and polypeptide fragments that specifically bind a novel isoform of CFR-1 that is expressed on neoplastic cells as well as on cells of pre-cancerous lesions, but not on normal cells. These polypeptides may be used in the therapy and diagnosis not only of tumors, but also of pre-cancerous structures both *in vitro* and *in vivo*. The novel isoform of CFR-1, fragments of this novel isoform, and nucleic acids encoding this novel isoform, may be used in methods of inducing an immune response against a neoplastic cell, in methods of generating

antibodies that specifically bind this novel isoform or fragments of this isoform, and in screening methods to identify additional therapeutic compounds.

Accordingly, the first aspect of the invention features an isolated polypeptide that specifically binds to a neoplastic cell or a cell of a pre-cancerous lesion, but does not specifically bind to a normal cell, where the normal cell is not a cell of the glomerular, fascicular zone of the adrenal gland or an epithelial cell of the collection tubes of the kidney. This isolated polypeptide may include amino acids 28-32, 51-53, and/or 90-100 of the sequence of SEQ ID NO:27. In desirable embodiments of the first aspect of the invention, the isolated polypeptide also includes amino acids 11-18, 36-43, and/or 82-104 of the sequence of SEQ ID NO:26. In a related aspect, the invention features an isolated polypeptide that includes amino acids 11-15, 30-46, and/or 79-88 of the sequence of SEQ ID NO:2 and/or amino acids 17-32, 48-54, and/or 87-95 of the sequence of SEQ ID NO:4, but does not include the full-length sequence of SEQ ID NO:2 or SEQ ID NO:4 and that that specifically binds to a neoplastic cell or a cell of a pre-cancerous lesion, but does not specifically bind to a normal cell, where the normal cell is not a cell of the glomerular, fascicular zone of the adrenal gland or an epithelial cell of the collection tubes of the kidney.

In other desirable embodiments, the polypeptide includes amino acids 11-18, 36-43, and/or 82-104 of SEQ ID NO:26 or amino acids 28-32, 51-53, and/or 90-100 of SEQ ID NO:27, but does not include the full-length amino acid sequence of SEQ ID NO:26 or SEQ ID NO:27.

In further desirable embodiments of the first aspects of the invention, the polypeptide is capable of inducing apoptosis of the neoplastic cell or the cell of the pre-cancerous lesion, but does not induce apoptosis of the normal cell. In addition, the neoplastic may be a Barrett's tumor cell or a cell of a tumor of the esophagus, stomach, intestine, rectum, liver, gallbladder, pancreas, lungs, bronchi, breast, cervix, prostate, heart, ovary, or uterus. Furthermore, the pre-cancerous lesion may be a dysplasia of the gastric mucosa, interstitial metaplasia of the stomach, inflammation of the gastric mucosa which is associated with the bacteria *Helicobacter pylori*, tubular and tubulovillous adenomas of the stomach, tubular adenoma of the colon, villous adenoma of the colon, dysplasia in ulcerative colitis, Barrett's dysplasia, Barrett's

metaplasia of the esophagus, cervical intraepithelial neoplasia I, cervical intraepithelial neoplasia II, cervical intraepithelial neoplasia III, squamous epithelial metaplasia, squamous epithelial dysplasia of the bronchus, low grade and high grade prostate intraepithelial neoplasia (PIN), breast ductal carcinoma in situ (D-CIS) or breast lobular carcinoma in situ (L-CIS).

In another desirable embodiment of the first aspects, the polypeptide is a functional fragment of an antibody, e.g., a V_L, V_H, F_V, F_C, Fab, Fab', or $F(ab')_2$ antibody fragment. In addition, the polypeptide may specifically bind to a polypeptide comprising SEQ ID NO:6 or a fragment thereof.

In the second aspect, the invention features an isolated nucleic acid molecule containing nucleic acids 31-54, 106-129, and/or 244-312 of the sequence of SEQ ID NO:28, and/or 82-96, 151-159, and/or 268-300 of the sequence of SEQ ID NO:29. In desirable embodiments of this aspect, the isolated nucleic acid molecule does not include the full-length sequence or SEQ ID NO:28 and/or SEQ ID NO:29. In a related aspect the invention features an isolated nucleic acid molecule containing nucleic acids 31-45, 88-138, and/or 235-264 of SEQ ID NO:1. Desirably, this nucleic acid molecule does not include the full-length sequence of SEQ ID NO:1. In the third aspect, the invention features an isolated nucleic acid molecule containing nucleic acids 49-96, 142-162, and/or 259-285 of SEQ ID NO:3. In a desirable embodiment of the third aspect of the invention, the nucleic acid molecule does not include the full-length sequence of SEQ ID NO:3.

In the fourth aspect, the invention features an isolated nucleic acid molecule including the sequence of SEQ ID NO:5 and in the fifth aspect, the invention features a vector containing the nucleic acid sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:28, and/or SEQ ID NO:29.

In the sixth aspect, the invention features an isolated cell, e.g., a mammalian cell, containing a vector that includes the nucleic acid sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:28, and/or SEQ ID NO:29.

In the seventh aspect, the invention features an isolated cell, e.g., a mammalian cell, that expresses the polypeptide of the first aspect of the invention. Desirably, the cell of the seventh aspect of the invention is a human cell.

In the eighth aspect, the invention features a method of producing the purified polypeptide of the first aspects of the invention. This method involves contacting a cell with a vector that includes SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:28, and/or SEQ ID NO:29 and isolating the polypeptide expressed by the vector.

The ninth aspect of the invention features a method of diagnosing a neoplasm or a pre-cancerous lesion in a mammal, e.g., a human. This method involves the steps of (a) contacting a cell or tissue sample derived from the mammal with a purified polypeptide of the first aspects of the invention, and (b) detecting whether the purified polypeptide specifically binds to the cell or tissue sample, where specific binding of the purified polypeptide to the cell or tissue sample is indicative of the mammal having a neoplasm or pre-cancerous lesion. In desirable embodiments of this aspect of the invention, the cell or tissue sample may be Barrett's tumors, tumors of the esophagus, stomach, intestine, rectum, liver, gallbladder, pancreas, lungs, bronchi, breast, cervix, prostate, heart, ovary, and uterus, dysplasia of the gastric mucosa, interstitial metaplasia of the stomach, inflammation of the gastric mucosa which is associated with the bacteria *Helicobacter pylori*, tubular and tubulovillous adenomas of the stomach, tubular adenoma of the colon, villous adenoma of the colon, dysplasia in ulcerative colitis, Barrett's dysplasia, Barrett's metaplasia of the esophagus, cervical intraepithelial neoplasia I, cervical intraepithelial neoplasia II, cervical intraepithelial neoplasia III, squamous epithelial metaplasia, squamous epithelial dysplasia of the bronchus, low grade and high grade prostate intraepithelial neoplasia (PIN), breast ductal carcinoma in situ (D-CIS) or breast lobular carcinoma in situ (L-CIS). In other desirable embodiments of the ninth aspect of the invention, the polypeptide is an antibody, such as murine antibody 58/47-69.

In further desirable embodiments of the ninth aspect, the polypeptide is conjugated to a detectable agent. This detectable agent may be a radionuclide, a fluorescent marker, an enzyme, a cytotoxin, a cytokine, or a growth inhibitor and the detectable agent may also be capable of inducing apoptosis of the cell. In addition, the polypeptide of the ninth aspect may be conjugated to a protein purification tag, e.g., a cleavable protein purification tag.

The tenth aspect of the invention features a method of treating a proliferative disorder in a mammal, for example, a human. This method involves the step of contacting a cell with the purified polypeptide of the first aspect, where binding of the purified polypeptide to the cell results in the induction of apoptosis of the cell. In desirable embodiments of this aspect, the polypeptide is an antibody, e.g., a humanized antibody, a chimeric antibody (i.e., one that comprises amino acid sequence derived from more than one species) or murine antibody 58/47-69. In addition, the polypeptide of the tenth aspect of the invention may be conjugated to a detectable agent. This detectable agent may be a radionuclide, a fluorescent marker, an enzyme, a cytotoxin, a cytokine, or a growth inhibitor. The polypeptide may also be conjugated to a protein purification tag, such as a cleavable protein purification tag.

In the eleventh aspect, the invention features a pharmaceutical composition that contains the isolated polypeptide of the first aspect in a pharmaceutically acceptable carrier and in the twelfth aspect, the invention features a diagnostic agent containing the isolated polypeptide of the first aspect of the invention.

In the thirteenth aspect, the invention features an isolated polypeptide, for example, one that is 90%, 95%, or 99% pure, that includes amino acids 469-518 of SEQ ID NO:6 and/or amino acids 739-748 of SEQ ID NO:6. In a desirable embodiment of the thirteenth aspect, the polypeptide does not include the full-length sequence of SEQ ID NO:6. In another desirable embodiment, the polypeptide of the thirteenth aspect is encoded by the nucleic acid sequence of SEQ ID NO:5.

In further desirable embodiments of the thirteenth aspect, the polypeptide is specifically bound by murine antibody 58/47-69 and/or includes a tumor-specific glycostructure. In addition, the polypeptide may have an apparent molecular weight of approximately 130 kD on a polyacrylamide gel and may be a polypeptide expressed by adenocarcinoma cell line 23132.

In other desirable embodiments of the thirteenth aspect of the invention, the polypeptide is expressed by a pre-cancerous lesion and not by normal cells of the same tissue type. This pre-cancerous lesion may be dysplasia of the gastric mucosa, interstitial metaplasia of the stomach, inflammation of the gastric mucosa which is associated with the bacteria *Helicobacter pylori*, tubular and tubulovillous adenomas

of the stomach, tubular adenoma of the colon, villous adenoma of the colon, dysplasia in ulcerative colitis, Barrett's dysplasia, Barrett's metaplasia of the esophagus, cervical intraepithelial neoplasia I, cervical intraepithelial neoplasia II, cervical intraepithelial neoplasia III, squamous epithelial metaplasia, squamous epithelial dysplasia of the bronchus, low grade and high grade prostate intraepithelial neoplasia (PIN), breast ductal carcinoma in situ (D-CIS) or breast lobular carcinoma in situ (L-CIS). Further, the polypeptide of the thirteenth aspect of the invention may be expressed by a tumor such as Barrett's tumor, or tumors of the esophagus, stomach, intestine, rectum, liver, gallbladder, pancreas, lungs, bronchi, breast, cervix, prostate, heart, ovary, or uterus, and not by a normal cell of the same tissue type.

The fourteenth aspect of the invention features a pharmaceutical composition that contains the polypeptide of the thirteenth aspect in a pharmaceutically acceptable carrier and the fifteenth aspect features a diagnostic agent including the isolated polypeptide of the thirteenth aspect.

The sixteenth aspect of the invention features a method of inducing a tumor-specific immune response in a mammal. This method includes the step of contacting the mammal, e.g., a human, with an isolated polypeptide containing the sequence of SEQ ID NO:6 or an isolated polypeptide that comprises amino acids 469-518 of SEQ ID NO:6 or amino acids 739-748 of SEQ ID NO:6 and does not comprise the full-length sequence of SEQ ID NO:6, where the contacting induces a tumor-specific immune response in the mammal. Desirably, the tumor-specific immune response involves the production of an antibody that induces apoptosis of a cell which is specifically bound by the antibody.

In further desirable embodiments of this aspect, the invention features a DNA vaccine comprising a plasmid vector or a viral vector which includes a nucleotide sequence encoding SEQ ID NO:6, or a fragment thereof, where, upon administration into a patient, a tumor-specific immune response is induced in the patient, e.g., a human patient. This immune response, for example, results in the formation of antibodies that specifically bind to a neoplasm or a pre-cancerous lesion.

In the seventeenth aspect, the invention features a method of producing an isolated polypeptide containing the sequence of SEQ ID NO:6 or a fragment thereof

that comprises amino acids 469-518 of SEQ ID NO:6 or amino acids 739-748 of SEQ ID NO:6 and does not comprise the full-length sequence of SEQ ID NO:6. This method involves the steps of (a) contacting a cell with a vector containing a nucleic acid sequence that is substantially identical or identical to SEQ ID NO:5 or a fragment thereof and (b) isolating the polypeptide expressed by the cell. In a desirable embodiment, the fragment comprises amino acids 469-518 of SEQ ID NO:6 and amino acids 739-748 of SEQ ID NO:6 and does not comprise the full-length sequence of SEQ ID NO:6.

The eighteenth aspect of the invention features a method of identifying a candidate therapeutic compound. This method involves the steps of (a) contacting a cell expressing a polypeptide containing the amino acid sequence of SEQ ID NO:6, e.g., adenocarcinoma cell line 23132 (DSMZ Accession No. DSM ACC 201), with a test compound and (b) determining whether the test compound induces apoptosis of the cell and not of a control cell contacted with the test compound, where a test compound that induces apoptosis of the cell and not of the control cell is a candidate therapeutic compound. In desirable embodiments of this method, fragments of SEQ ID NO:6, for example, ones that comprise amino acids 469-518 of SEQ ID NO:6 or amino acids 739-748 of SEQ ID NO:6 and do not comprise the full-length sequence of SEQ ID NO:6, may be used.

In another aspect, the invention features a method of producing an antibody that specifically binds to a neoplastic cell. This method involves (a) administering a purified polypeptide having amino acids 469-518 of SEQ ID NO:6 and/or amino acids 739-748 of SEQ ID NO:6 and not including the full length sequence of SEQ ID NO:6 to a mammal and (b) isolating from the mammal, an antibody that specifically binds to the polypeptide having amino acids 469-518 of SEQ ID NO:6 and/or amino acids 739-748 of SEQ ID NO:6. Alternatively, the purified polypeptide administered to the mammal may comprise the full-length sequence of SEQ ID NO:6. In a desirable embodiment of this aspect of the invention, the polypeptide is purified from adenocarcinoma cell line 23132 (DSMZ Accession No. DSM ACC 201). In other desirable embodiments, the polypeptide having amino acids 469-518 of SEQ ID NO:6 and/or amino acids 739-748 of SEQ ID NO:6 includes a tumor-specific

glycostructure. In another desirable embodiment of this aspect, the method also involves isolating a cell expressing the antibody from the mammal.

Definitions

By "novel CFR-1 isoform" and "the isoform of CFR-1 that is recognized by a PAM-1 antibody" is meant an isoform of CFR-1 that includes amino acids 469-518 of SEQ ID NO:6 and/or amino acids 739-748 of SEQ ID NO:6 and that is expressed by neoplastic cells and cells of a pre-cancerous lesion and not by a normal cell, where cells of the glomerular, fascicular zone of the adrenal gland or an epithelial cell of the collection tubes of the kidney are excluded from the group of normal cells. Desirably, the novel CFR-1 isoform does not comprise the full-length amino acid sequence of SEQ ID NO:6. In further desirable embodiments, the novel CFR-1 isoform is specifically-bound by human monoclonal antibody 103/51 and/or murine antibody 58/47-69 and has a molecular mass of approximately 130 kD. The use of the term "approximately" reflects that one skilled in the art would recognize that these types of size determinations are affected by changes or variations of the methods of the molecular size determination, e.g., gel electrophoresis conditions. In addition, the novel CFR-1 isoform is a polypeptide present on adenocarcinoma cell line 23132 (DSMZ Accession No. DSM ACC 201).

In other desirable embodiments, the novel CFR-1 isoform is expressed by the cells of the following types of pre-cancerous lesion, but not normal cells: *H. pylori* induced gastritis, intestinal metaplasia and dysplasia of the stomach, ulcerative colitis-related dysplasia and adenomas of the colon, Barrett metaplasia and dysplasia of the esophagus, squamous cell metaplasia and dysplasia of the lung, cervical intraepithelial neoplasia, low grade and high grade prostate intraepithelial neoplasia (PIN), breast ductal carcinoma in situ (D-CIS) and breast lobular carcinoma in situ (L-CIS).

In further desirable embodiments, the novel CFR-1 isoform is expressed by carcinomas of the following tissues, but not normal cells of the same tissues: esophagus, stomach, colon, liver, pancreas, lung, breast, ovary, uterus, cervix, and prostate.

By a "PAM-1 antibody" is meant a polypeptide that specifically binds to the isoform of CFR-1 that comprises the amino acid sequence of SEQ ID NO:6 and that is expressed by 23132 cells. In a desirable embodiment, a PAM-1 antibody binds a tumor-specific glycostructure of the CFR-1 isoform having the amino acid sequence of SEQ ID NO:6. For example, a PAM-1 antibody may be human monoclonal antibody 103/51, murine antibody 58-49/69, or a humanized or chimeric antibody containing all or part of the sequence of SEQ ID NO:2 and/or 4. In further desirable embodiments, a PAM-1 antibody can induce apoptosis or alter proliferation, or both, in a neoplastic cell or a cell of a pre-cancerous lesion, but not a normal cell. In additional desirable embodiments, a PAM-1 antibody comprises the amino acid sequence of SEQ ID NO:2 and/or SEQ ID NO:4 or is encoded, in part, by the nucleic acid sequence of SEQ ID NO:1 and/or SEQ ID NO:3. In further desirable embodiments, a PAM-1 antibody may comprise amino acids 11-18, 36-43, and/or 82-104 of SEQ ID NO:26 and/or amino acids 28-32, 51-53, and/or 90-100 of SEQ ID NO:27.

By a "functional fragment" or a "fragment of a PAM-1 antibody" as used herein in reference to polypeptide, is meant a fragment that retains at least one biological activity of the full-length polypeptide. Examples of such a biological activity are the ability to specifically bind an antigen, induce apoptosis, and/or inhibit cell proliferation. The biological activities of a functional fragment may be determined, for example, using any one of the assays described herein.

Examples of functional fragments of an antibody are V_L, V_H, F_V, F_C, Fab, Fab', or F(ab')$_2$ fragments which are known to one skilled in the art (see, e.g., Huston et al., Cell Biophys. 22:189-224, 1993; and Harlow and Lane, Using Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, N.Y., 1999). Desirably, a "functional fragment" has an amino acid sequence that is substantially identical to a fragment, e.g., 3, 4, 5, 10, 15, 20, 15, 30, 50, 75, or 100 contiguous amino acids, of the amino acid sequence of SEQ ID NO:2 , SEQ ID NO:4, SEQ ID NO:26, or SEQ ID NO:27. In more desirable embodiments, a "functional fragment" is identical to a fragment of the sequence of SEQ ID NO:2 , SEQ ID NO:4, SEQ ID NO:26, or SEQ ID NO:27. Such a "functional fragment" may contain 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 15,

30, 50, 75, or 100 contiguous amino acids of SEQ ID NO:2 , SEQ ID NO:4, SEQ ID NO:26, or SEQ ID NO:27, or may be the entire amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:26, or SEQ ID NO:27. In desirable embodiments, such a fragment includes one or more of the Complement Determining Regions (CDR) of the V_H or the V_L regions of the murine PAM-1 antibody 58-49/69 or human PAM-1 antibody 103/51. For example, a functional fragment may include amino acids 11-15, 30-46, and/or 79-88 of SEQ ID NO:2; and/or amino acids 17-32, 48-54, and/or 87-95 of SEQ ID NO:4. Other examples of functional fragments include polypeptides having amino acids 11-18, 36-43, and/or 82-104 of SEQ ID NO:26 and/or amino acids 28-32, 51-53, and/or 90-100 of SEQ ID NO:27.

By "detectable agent" is meant a compound that is linked to a diagnostic agent to facilitate detection. Such a "detectable agent" may be covalently or non-covalently linked to a diagnostic agent. In addition, the linkage may be direct or indirect. Examples of "detectable agents" include, protein purification tags, cytotoxins, enzymes, paramagnetic labels, enzyme substrates, co-factors, enzymatic inhibitors, dyes, radionuclides, chemiluminescent labels, fluorescent markers, growth inhibitors, cytokines, antibodies, and biotin.

By a "diagnostic agent" is meant a compound that may be used to detect a neoplastic cell by employing any one of the assays described herein as well as any other method that is standard in the art. A diagnostic agent may include, for example, an antibody which specifically binds to cell line 23132 (DSMZ Accession No. ACC 201), but not to normal cells. In addition, a diagnostic agent may specifically bind to cells of pre-cancerous lesions such as those exhibiting *H. pylori* induced gastritis, intestinal metaplasia and dysplasia of the stomach, ulcerative colitis-related dysplasia and adenomas of the colon, Barrett metaplasia and dysplasia of the esophagus, squamous cell metaplasia and dysplasia of the lung, cervical intraepithelial neoplasia, low grade and high grade prostate intraepithelial neoplasia (PIN), breast ductal carcinoma in situ (D-CIS) and breast lobular carcinoma in situ (L-CIS). A diagnostic agent may also specifically bind carcinomas of the esophagus, stomach, colon, liver, pancreas, lung, breast, ovary, uterus, cervix, and prostate, but not non-neoplastic cell of the same tissue type. Furthermore, a "diagnostic agent" may alter cell proliferation,

induce apoptosis, or both only when it is bound to a neoplastic cell or a cell of a pre-cancerous lesion, but not a normal cell. Moreover, a "diagnostic agent" may include, for example, peptides, polypeptides, synthetic organic molecules, naturally-occurring organic molecules, nucleic acid molecules, and components thereof, as well as one or more detectable agent covalently or non-covalently linked to the diagnostic agent.

By "high stringency hybridization conditions" is meant, for example, hybridization at approximately 42°C in about 50% formamide, 0.1 mg/ml sheared salmon sperm DNA, 1% SDS (Sodium Dodecyl Sulfate), 2X SSC (Sodium Citrate Buffer), 10% Dextran Sulfate, a first wash at approximately 65°C in about 2X SSC, 1% SDS, followed by a second wash at approximately 65°C in about 0.1X SSC. Alternatively, "high stringency hybridization conditions" may include hybridization at approximately 42°C in about 50% formamide, 0.1 mg/ml sheared salmon sperm DNA, 0.5% SDS, 5X SSPE, 1X Denhardt's, followed by two washes at room temperature in 2X SSC, 0.1% SDS, and two washes at between 55-60°C in 0.2X SSC, 0.1% SDS.

"Altering cell proliferation," as used herein, refers to a reduction or an increase in the rate of cell division of a cell in comparison with the normal rate of cell division of that type of cell under the same conditions. Cell proliferation may be assayed using a number of methods standard in the art, for example, the MTT cell proliferation assay described herein, BrdU incorporation, and ^{3}H thymidine uptake. Such assays are described, for example, in Ausubel *et al., Current Protocols in Molecular Biology*, Wiley Interscience, New York, 2001; and Sambrook et al., *Molecular Cloning: A Laboratory Manual*, 3rd edition, Cold Spring Harbor Laboratory Press, N.Y., 2001. Desirably, the increase or decrease of cell proliferation is 20%, 40%, 50%, or 75%. In desirable embodiments, the increase or decrease of cell proliferation is 80%, 90%, 95%. In another desirable embodiment cell proliferation is completely inhibited.

"Inducing apoptosis," as used herein, refers to the appearance of characteristics in a cell that are well defined in the art (see, e.g., Wyllie et al., Br. J. Cancer 80 Suppl. 1:34-37, 1999; Kerr et al., Br. J. Cancer 26:239-257, 1972). These characteristics include morphological characteristics, such as membrane blebbing, DNA condensation, as well as changes in F-actin content, mitochondrial mass, and

membrane potential. The induction of apoptosis may be assayed using a number of methods standard in the art, for example, a cell death ELISA, TUNEL staining, DNA stains, e.g., Hoechst 33258, and staining with various vital dyes such as acridine orange, Mito Tracker Red® staining (Molecular Probes, Eugene, OR), and Annexin V® staining (Becton Dickinson, NJ). As used herein "inducing apoptosis" refers to an increase in the number of cells undergoing apoptosis when compared with a control cell population under the same conditions. For instance, the increase of apoptosis may be 10%, 20%, 40%, 50%, or 75%. In desirable embodiments, the induction of apoptosis results in an increase of apoptosis that is 2-fold, 3-fold, 10-fold, or even 100-fold over that seen in a control cell population.

A "humanized antibody" as used herein, is a genetically engineered antibody in which a minimum of a non-human, e.g., a murine, antibody sequence is combined with human antibody sequence and still maintains the binding specificity of the original non-human antibody. In desirable embodiments, a humanized antibody contains 15%, 20%, 25%, 30%, or 40% non-human sequence. In more desirable embodiments, a humanized antibody contains 5% or 10% non-human sequence. In addition, a humanized antibody desirably induces no or only a minimal human immune response.

A "neoplastic cell," as used herein, refers to a cell which is undergoing cell division, not undergoing apoptosis, or both, under inappropriate conditions. For example, a "neoplastic cell" may undergo cell division when a corresponding normal cell does not undergo cell division, or, alternatively, a "neoplastic cell" may not respond to normal cell-cycle checkpoint controls.

By a "cell of a pre-cancerous lesion" is meant cells that are undergoing cell division, not undergoing apoptosis, or both, under inappropriate conditions, but that have not developed into a cancerous tumor. For example, cells in a pre-cancerous lesion may undergo cell division when a corresponding normal cell does not undergo cell division, or, alternatively, cells of a pre-cancerous lesion may not respond to normal cell-cycle checkpoint controls. Examples of pre-cancerous lesions include *H. pylori* induced gastritis, intestinal metaplasia and dysplasia of the stomach, ulcerative colitis-related dysplasia and adenomas of the colon, Barrett metaplasia and dysplasia

of the esophagus, squamous cell metaplasia and dysplasia of the lung, cervical intraepithelial neoplasia, low grade and high grade prostate intraepithelial neoplasia (PIN), breast ductal carcinoma in situ (D-CIS) and breast lobular carcinoma in situ (L-CIS).

By a "normal cell" as used herein is meant a cell that is neither a neoplastic cell nor a cell of a pre-cancerous lesion.

A "proliferative disease," as used herein, refers to any disorder that results in the abnormal proliferation of a cell. Specific examples of proliferative diseases are various types of neoplasms, such as stomach adenocarcinoma, colorectal adenocarcinoma, lung adenocarcinoma, and adenocarcinoma of the pancreas. However, proliferative diseases may also be the result of the cell becoming infected with a transforming virus.

A "protein purification tag," as used herein, is a peptide, e.g., an epitope tag, that is covalently or non-covalently added to a protein to aid in the purification of the protein. Desirably such peptides bind with high affinity to an antibody or to another peptide such as biotin or avidin. Commercially available examples of epitope tags include His-tags, HA-tags, FLAG®-tags, and c-Myc-tags. However, any epitope that is recognized by an antibody also may be used as a protein purification tag. See, for example, Ausubel *et al., Current Protocols in Molecular Biology*, Wiley Interscience, New York, 2001; and Sambrook et al., *Molecular Cloning: A Laboratory Manual*, 3rd edition, Cold Spring Harbor Laboratory Press, N.Y., 2001. Protein purification tags may be cleaved from a protein, for example, by using an enzyme, e.g., thrombin, or a chemical, e.g., cyanogen bromide.

By "specifically binds" and "specifically recognizes" as used herein in reference to a polypeptide, e.g., an antibody, is meant an increased affinity of a polypeptide for a particular protein, e.g., an antigen, relative to an equal amount of any other protein. For example, an antibody, e.g., the human or murine PAM-1 antibody, that specifically binds to 23132 cells desirably has an affinity for its antigen that is least 2-fold, 5-fold, 10-fold, 30-fold, or 100-fold greater than for an equal amount of any other antigen, including related antigens. Binding of a polypeptide to another

polypeptide may be determined as described herein, and by any number of standard methods in the art, e.g., Western analysis, ELISA, or co-immunoprecipitation.

By "substantially identical" is meant a polypeptide or nucleic acid exhibiting at least 80%, 85%, 90%, or 95% identity to a reference amino acid (e.g., the sequence of SEQ ID NO:2, 4, 6, 26, or 27) or nucleic acid sequence (e.g., the sequence of SEQ ID NO:1, 3, 5, 28, or 29), or a fragment thereof. In desirable embodiments, the polypeptide or nucleic acid sequence is at least 98%, 99%, 99.4%, 99.5%, 99.6 %, 99.7%, 99.8%, 99.9%, or even 100% identical to a reference amino acid or nucleic acid sequence. For polypeptides, the length of comparison sequences will generally be at least 3, 4, 5, 6, 8, 10, or 15 amino acids and desirably at least 20 or 25 contiguous amino acids. In more desirable embodiments, the length of comparison sequences is at least 30, 50, 75, 90, or 95 contiguous amino acids, or even the full-length amino acid sequence. For nucleic acids, the length of comparison sequences will generally be at least 9, 10, 12, 15, 18, 20, 24, or 25 contiguous nucleotides, and desirably at least 30 contiguous nucleotides. In more desirable embodiments, the length of comparison sequences is at least 50, 75, 150, 225, 270, 280, 285, or 290 contiguous nucleotides, or even the full-length nucleotide sequence.

Sequence identity may be measured using sequence analysis software on the default setting (e.g., Sequence Analysis Software Package of the Genetics Computer Group, University of Wisconsin Biotechnology Center, 1710 University Avenue, Madison, WI 53705). Such software may match similar sequences by assigning degrees of homology to various substitutions, deletions, and other modifications. Conservative substitutions typically include substitutions within the following groups: glycine, alanine, valine, isoleucine, leucine; aspartic acid, glutamic acid, asparagine, glutamine; serine, threonine; lysine, arginine; and phenylalanine, tyrosine.

Multiple sequences may also be aligned using the Clustal W(1.4) program (produced by Julie D. Thompson and Toby Gibson of the European Molecular Biology Laboratory, Germany and Desmond Higgins of European Bioinformatics Institute, Cambridge, UK) by setting the pairwise alignment mode to "slow," the pairwise alignment parameters to include an open gap penalty of 10.0 and an extend gap penalty of 0.1, as well as setting the similarity matrix to "blosum." In addition,

the multiple alignment parameters may include an open gap penalty of 10.0, an extend gap penalty of 0.1, as well as setting the similarity matrix to "blosum," the delay divergent to 40%, and the gap distance to 8.

By "purified" or "isolated" is meant separated from other components that naturally accompany it. Typically, a factor is "purified" or "isolated" when it is at least 50%, by weight, free from proteins, antibodies, and naturally-occurring organic molecules with which it is naturally associated, or in reference to a nucleic acid molecule, is free from the nucleic acid sequences that naturally flank the sequence of the nucleic acid molecule in the genome of an organism. Desirably, the factor is at least 75%, more desirably, at least 90% or 95% and most desirably, at least 99%, by weight, pure. A substantially pure factor may be obtained by chemical synthesis, separation of the factor from natural sources, or production of the factor in a recombinant host cell that does not naturally produce the factor. Proteins, vesicles, and organelles may be purified by one skilled in the art using standard techniques, such as those described by Ausubel *et al.* (*Current Protocols in Molecular Biology*, Wiley Interscience, New York, 2001). The factor is desirably at least 2, 5, or 10 times as pure as the starting material, as measured using polyacrylamide gel electrophoresis, column chromatography, optical density, HPLC analysis, or Western analysis (Ausubel *et al., Current Protocols in Molecular Biology*, Wiley Interscience, New York, 2001). Desirable methods of purification include immunoprecipitation, column chromatography such as immunoaffinity chromatography and nickel affinity columns, magnetic bead immunoaffinity purification, and panning with a plate-bound antibody.

By a "tumor-specific glycostructure" is meant a glycostructure on a polypeptide which is present on a neoplastic cell or a pre-cancerous cell, but not on a normal cell of the same cell type.

By "vector" or "expression vector" is meant an expression system, a viral vector, a nucleic acid-based shuttle vehicle, a nucleic acid molecule adapted for nucleic acid delivery, or an autonomous self-replicating circular DNA (e.g., a plasmid). When a vector is maintained in a host cell, the vector can either be stably replicated by the cell during mitosis as an autonomous structure, incorporated into the genome of the host cell, or maintained in the host cell's nucleus or cytoplasm.

Advantages

The CFR-1 isoform described herein, as well as antibodies that bind this isoform, unlike previously described proliferation markers, e.g., Ki67, may be used to differentiate between normal and neoplastic cells. In addition, the polypeptides of the invention can be used to identify cells that are likely to give rise to a tumor, such as cells of a pre-cancerous lesion.

Other features and advantages of the invention will be apparent from the following Detailed Description, the drawings, and the claims.

Brief Description of the Drawings

Fig. 1A is an image of a protein gel and shows the antigen recognized by antibody 103/51. Membrane fractions from stomach carcinoma cell line 23132 were purified using chromatographic procedures and whole membrane fraction (lane 2), or purified proteins (lane 3) were stained with Coomassie (lane 1: 10 kDa ladder). Western blot analysis with antibody 103/51 on membrane fractions of cell line 23132 showed one reaction with a protein with a molecular mass of approximately 130 kD (lane 4). Specificity of processed membrane extracts was controlled by Western blotting with 103/51 (lane 5). The protein band indicated by the arrow was excised from a preparative gel and used for MALDI mass mapping and immunization of mice.

Fig 1B is a spectrograph of the identification of the 130 kDa gel-separated protein by high resolution MALDI peptide mass mapping. Peaks labeled with '*' match the calculated masses of tryptic peptides of U28811 human cysteine-rich fibroblast growth factor receptor (CFR-1) with a mass accuracy better than 50 ppm. Peaks labeled with 'T' correspond to trypsin autolysis products. The inset shows the mass resolution ($m/\Delta m = 9000$) of the peak at m/z 1707.818.

Figs. 2A - 2F are a series of images showing the effect of CFR-1 antisense transfection on antibody 103/51 staining and live cell staining (Magnification 200x). **Fig. 2A** shows cell line 23132 transiently transfected with a control vector and, after acetone fixation, shows intensive staining with antibody 103/51. **Fig. 2B** shows reduced staining in cells transiently transfected with a CFR-1 antisense vector. To reduce background staining in immunohistochemical staining, live cell staining was

performed with cell line 23132. A clear membrane staining is visible (**Fig. 2C**). **Fig. 2D** shows control live cell staining, using only secondary antibody, on cell line 23132. **Fig. 2E** shows a lack of live cell staining on cell line Colo-699 with antibody 103/51, which indicates that this cell line is negative for expression of CFR-1. **Fig. 2F** shows control live cell staining, using only secondary antibody, on cell line Colo-699.

Fig. 2G is a graph of flow cytometry results of cell line 23132 with antibodies Chromopure human IgM (grey) and 103/51.

Fig. 2H is a graph of the analysis of cells transfected with control vector pHOOK-2 with flow cytometry 48 hours after transfection.

Fig. 2I is a graph of cells transfected with CFR-1 antisense vector, which shows a clear decrease in binding of antibody 103/51.

Figs. 3A and **3B** are images showing the effect of deglycosylation on staining with antibody 103/51. **Fig. 3A** shows 23132 cells incubated with deglycosylation buffer and acetone-fixed show intense staining with antibody 103/51 and **Fig. 3B** shows 23132 cells treated with N-glycosidase followed by acetone fixation. A clear reduction in staining is evident as a result of the N-glycosidase treatment.

Fig. 3C is a Western blot showing the effect of deglycosylation of membrane extracts of cell line 23132 on the reaction with antibody 103/51. Extracts which were incubated for 16 hours with deglycosylation buffer (Buffer) show no difference in staining in comparison with untreated extracts (Control). In contrast, incubation with N-glycosidase leads to a clear reduction in staining (N-glyco).

Figs. 4A-4D are a series of images showing immunohistochemical staining with murine antibody 58/47-69 and 103/51 on stomach adenocarcinoma cells. To show identical specificity of antibody 103/51 and murine antibody 58/47-69, diffuse-type stomach adenocarcinoma was stained with haematoxilin-eosin (**Fig. 4A**), antibodies 103/51 (**Fig. 4B**) and 58/47-69 (**Fig. 4C**), and anti-cytokeratin 18 as a positive control. Identical staining in **Fig. 4C** and **Fig. 4D** indicates identical specificity. (The arrows point to tumor cells.)

Figs. 5A-5D are a series of images showing immunohistochemical staining of antibody 103/51 on different gastric tissues. Cryo-sections of gastric tissues were stained with haematoxilin-eosin ("HE"), antibody Ki67 (to indicate proliferating cells)

and antibody 103/51. The magnification is 100x. **Fig. 5A** shows gastric tissue with inflammation. **Fig. 5B** shows *H. pylori* induced gastritis (inserts show a higher magnification of marked glands). **Fig. 5C** shows dysplasia and **Fig. 5D** shows gastric adenocarcinoma cells.

Figs. 6A-6F are a series of images of immunohistochemical staining with antibody 103/51 on different cancerous and normal tissues. The staining of antibody 103/51 on the following tissues is shown: Carcinoma of the ampulla of Vater (**Fig. 6A**), mamma carcinoma invasive lobular (**Fig. 6B**), adenocarcinoma of the colon and no staining of normal goblet-cell epithelium of the colon (**Fig. 6C**), hepatocellular carcinoma (**Fig. 6D**), glomerular and fascicular zones of the adrenal gland (**Fig. 6E**), collection tubes of the kidney-specific staining of the Golgi apparatus (arrow) (**Fig. 6F**). Arrows in Figs. **6A-6D** indicate tumor cells, the arrow in **Fig. 6C** points to goblet cells, the arrow in (**Fig. 6F**) indicates Golgi apparatus (Magnification 400x (**Figs. 6A-6E**) and 200x (**Fig. 6F**)).

Figs. 7A-7D are a series of graphs showing stimulation of cell lines with antibodies 103/51 and 58/47-69 as determined by colorimetric MTT-assays. **Fig. 7A** is graph of a titration with purified antibody 103/51 which shows an increase in stimulation up to 4 µg/ml. Higher concentrations do not lead to higher stimulation (c = Control, no antibody added). **Fig. 7B** is a graph of the results of an MTT-assay with equal concentrations (4 µg/ml) of purified antibodies 103/51 and 58/47-69 and shows comparable stimulation of tumor cell 23132 after one or two days of incubation (Control 1 = chromopure human IgM, Control 2, uncorrelated mouse IgM). **Fig. 7C** is a graph of the results of an experiment in which cell line 23132 was transiently transfected with control vector pHOOK-2 or CFR-1 antisense vector, incubated for 24 hours, and tested in the MTT assay for stimulation with 4 µg/ml purified antibody 103/51 after 24 hours. Untransfected cells were also incubated as control (Control, uncorrelated human IgM). Fig. 7D is a graph of the results of an MTT-assay with equal concentrations (4 µg/ml) of antibody 103/51 on different epithelial tumor cell lines. This graph shows stimulation only on the cell line 23132 24 hours after addition of antibody. Cell lines that do not express the antigen recognized by the

PAM-1 antibody (Colo-699 and EPLC-272H) do not show any stimulation by antibody 103/51.

Figs. 8A and **8B** are the nucleic acid sequence (SEQ ID NO:1) and the amino acid sequence (SEQ ID NO:2) of the variable region of the heavy chain of murine antibody NM58-49/69. The J-gene and D-gene, as well as Complement Determining Regions (CDR)1 to 3 are indicated in **Fig. 8B**.

Figs. 9A and **9B** are the nucleic acid sequence (SEQ ID NO:3) and the amino acid sequence (SEQ ID NO:4) of the variable region of the light chain of murine antibody NM58-49/69. Complement Determining Regions (CDR)1 to 3 are indicated in **Fig. 9B**.

Figs. 10-1 to **10-5** are the nucleic acid (SEQ ID NO:5) and amino acid sequence (SEQ ID NO:6) of the novel isoform of CFR-1 expressed by cell line 23132.

Figs. 11A and **11B** are a series of Coomassie blue stained SDS gels and Western blots showing fragments of antibody PAM-1. Intact PAM-1 antibody was digested with pepsin to yield antibody fragments. PAM-1 antibody was digested with Pepsin to yield antibody fragments. **Fig. 11A** shows a Coomassie blue stained SDS gel of intact PAM-1 antibody (lane 1) and the fragmented antibody after 10 min (lane 2) and 15 min (lane 3) of pepsin treatment. **Fig. 11B** shows the specific protein bands of intact (lane 1) and fragmented (lane 2 and 3) PAM-1 antibody.

Figs. 12A and **12B** are a series of images showing immunohistochemical staining of intact and fragmented PAM-1 antibody on pre-malignant and malignant tissue. Paraffin sections were stained with positive control (anti-cytokeratin 8), unrelated fragmented IgM as a negative control, intact PAM-1 antibody or fragmented PAM-1 antibody. **Fig. 12A** shows vilous adenoma of the colon (precursor lesion of coloncarcinoma); and **Fig 12 B** shows gastric adenocarcinoma. The original magnification was 100x.

Figs. 13A and 13B are graphs showing functional analyses of fragmented PAM-1 antibody *in vitro*. The consequences of fragmented PAM-1 antibody treatment on the proliferation of stomach cancer cell line 23132/87 was measured by MTT proliferation assay (**Fig 13A**) and Cell Death Detection ELISA^{PLUS} apoptosis assay (**Fig 13B**). **Fig 13A** shows concentration dependent inhibition of cell

proliferation with fragmented PAM-1 antibody. **Fig. 13B** shows antibody induced apoptosis with the fragmented form of PAM-1 after 24 hours of treatment (control 1: no antibody was added; control 2: unrelated fragmented IgM was added at a similar concentration).

Figs. 14A-14C are a series of graphs showing the results of *in vivo* experiments with human carcinoma cells treated with fragmented PAM-1 antibody in NMRI nu/nu mice. **Fig. 14A** shows tumor size development of mice treated with fragmented form of PAM-1 antibody or unrelated fragmented IgM (control group) over a period of 23 days. **Figs. 14B** and **14C** show that antibody-reduced tumor mass is measurable in mice treated with fragmented PAM-1 antibody after 23 days. A group of mice treated with unrelated fragmented IgM served as a control in all experiments.

Figs. 15A-15F are a series of images showing immunoperoxidase staining of paraffin-embedded tumor sections with Klenow FragEL DNA fragmentation Kit for apoptosis. Sections were obtained from mice treated with either unrelated fragmented IgM (**Figs. 15A-15C**) or fragmented PAM-1 antibody (**Figs. 15D-15F**). **Figs. 15A** and **15D** show HE staining; **Figs. 15B** and **15E** show immunoperoxidase staining with apoptosis positive control, all cell nuclei are stained; and Figs. **15C** and **15F** show immunoperoxidase staining with DNA fragmentation Kit, only the nuclei of apoptotic cells are stained (original magnification, x100/ x200).

Fig. 16 is a comparison of the amino acid sequence of the CFR-1 obtained from cell line 23132 (SEQ ID NO:6) to the published sequences of CFR-1 and MG160 (SEQ ID NOS:7 and 8). These experimental comparisons primarily show that the CFR-1 protein obtained from cell line 23132 is not identical to the CFR-1 sequences previously known, but represents a novel isoform thereof. The differences between the sequences are underlined.

Fig. 17 is the nucleic acid sequence (SEQ ID NO:26) and the amino acid sequence (SEQ ID NO:28) of the variable region of the heavy chain of the human PAM-1 antibody (clone 103/51). The Complement Determining Regions (CDR)1 to 3 are indicated.

Fig. 18 is the nucleic acid sequence (SEQ ID NO:27) and the amino acid sequence (SEQ ID NO:29) of the variable region of the light chain of the human PAM-1 antibody (clone 103/51). The Complement Determining Regions (CDR)1 to 3 are indicated.

Figs. 19A and **19B** are a series of graphs showing that a PAM-1 recombinant IgG antibody induces both a reduction of tumor volume (**Fig. 19A**) and tumor weight (**Fig. 19B**) of stomach cancer *in vivo* using a mouse model. Mice receiving the control IgG antibody (Chrompure human IgG; "CP IgG") are indicated by shaded circles and mice receiving the PAM-1 Ab are indicated by open circles.

Figs. 20A and **20B** are a series of graphs showing that a PAM-1 recombinant IgG antibody induces both a reduction of tumor volume (**Fig. 20A**) and tumor weight (**Fig. 20B**) of pancreatic cancer *in vivo* using a mouse model. Mice receiving the control IgG antibody (Chrompure human IgG; "CP IgG") are indicated by shaded circles and mice receiving the PAM-1 Ab are indicated by open circles.

Figs. 21A - 21D are a series of images showing keratin staining (**Figs. 21A and 21C**) and haematoxilin-eosin ("H & E") staining (**Figs. 21B and 21D**) of pancreatic cancer in mice injected either with a control IgG antibody (**Figs. 21A and 21B**) or PAM-1 IgG (**Figs. 21C and 21D**). A reduction of keratin staining in **Fig. 21C** indicates tumor cell death and apoptotic cells are indicated by arrows in **Fig. 21D**.

Detailed Description

The present invention features novel polypeptides and methods of using these polypeptides in the diagnosis, detection, monitoring, and treatment of neoplasms in a mammal, e.g., a human. In particular, the isoform of CFR-1 described herein and polypeptides that bind this antigen, such as human monoclonal antibody 103/51 and murine antibody 58-49/69, enable the therapy and diagnosis not only of tumors, but also of pre-cancerous structures both *in vitro* and *in vivo*.. Moreover, the novel isoform of CFR-1 may be used in screening methods to identify additional therapeutic compounds.

Immunological studies have shown that in cases in which the immune system cannot effectively combat malignant cells, cellular and humoral activity is measurable, but is not sufficient to destroy the tumor cells. Currently, an effective approach to increase the efficacy of the immune response is to isolate the antibodies arising from the immune response of the patient, reproduce them in a suitable way, and use them therapeutically. Thus, for example, antibodies originating from patients having lung, esophageal, and colon cancers may be isolated and human monoclonal antibodies may be derived therefrom, which, for example, directly influence differentiation and growth of the tumor cells.

We have described that the human antibody 103/51 (human PAM-1), which was isolated from a stomach cancer patients with diffuse-type adenocarcinoma and cross-reacts with cells present in *H. pylori*-induced gastritis and stomach cancer cells. Sequencing of the antibody variable gene regions identified the human PAM-1 antibody (103/51) as an autoreactive antibody. In low doses, PAM-1 antibodies (both human and murine) have a mitotic effect on stomach cancer cells *in vitro*, in which they bind on a 130 kD membrane receptor.

The cellular receptor of monoclonal antibody 103/51 was previously unknown. In the course of the experiments leading to the present invention, we were able to identify this cellular receptor. The monoclonal antibody 103/51 reacts with its receptor during Western blot analysis only under very specific stringency conditions and non-specific reactions are found with an array of further proteins, caused by denaturing artifacts.

Sequencing analyses have shown that the antigen recognized by PAM-1 is homologous to the CFR-1 protein, but is not identical to this protein. This isoform of CFR-1 is specifically bound by human PAM-1 antibody (103/51) and/or murine PAM-1 antibody (58/47-69). The isoform of CFR-1 described herein is specific for tumor cells, particularly for gastric carcinoma cells and is not expressed by normal stomach tissue.

We further characterized this isoform of CFR-1 and determined that it contains a special glycostructure that is linked to the protein backbone via an N-linkage. In addition, the specificity for the antigen of human PAM-1 antibody 103/51 was

confirmed by producing murine antibodies from purified molecules having identical reactions and functions, through immunohistochemical staining, and an MTT assay of two cell lines that do not express the CFR-1 isoform recognized by the PAM-1 antibody. The isoform of the CFR-1 molecule, which was detected by both the human and the murine antibodies, is localized in the cell membranes of the epithelial cells, and has an expression pattern which differs from that previously described for wild-type CFR-1 (Burrus *et al.*, 1992, Mol. Cell. Biol. 12:5600-5609).

CFR-1

Wild-type CFR-1, which was isolated as a high-affinity FGF-binding protein from chicken fibroblasts (Burrus *et al.*, 1992, Mol. Cell. Biol. 12:5600-5609), binds to a number of FGFs and may have a role in the regulation of cellular proliferation. In Chinese hamster ovary cells (CHO), CFR-1 was found to be expressed only in the Golgi apparatus (Burrus *et al.*, 1992, Mol. Cell. Biol. 12:5600-5609), but it can also be secreted in a mutant form (Zuber *et al.*, 1997, J. Cell Physiol. 170:217-227). Depending on the organism, two detected variants of CFR-1, ESL-1, and MG-160 share sequence homologies between 80% and 95% (Burrus *et al.*, 1992, Mol. Cell. Biol. 12:5600-5609; Stieber *et al.*, 1995, Exp. Cell Res. 219:562-570; Steegmaier *et al.*, 1995, Nature 373:615-620; Mourelatos *et al.*, 1996, DNA Cell Biol. 15:1121-1128) and do not appear to share any sequence homologies to other known proteins. The function and cellular distribution of CFR-1 and the ESL-1 and MG-160 is relatively undefined and contradictory. It has been shown that MG-160, which is a medial Golgi sialoglycoprotein and was purified from rat brains, plays a role in intracellular FGF trafficking (Zuber *et al.*, 1997, J. Cell Physiol. 170:217-227).

Recent findings have shown that the localization of this protein is not restricted to the Golgi apparatus. However, if truncated at the c-terminus, the protein can be localized to the plasma membrane and filopodia (Gonatas *et al.*, 1998, J. Cell Sci. 111:249-260). This is consistent with the finding that the third homologue, ESL-1, which was isolated from mouse neutrophilic progenitor cells (32Dcl3), is located in the Golgi apparatus as well on the cell surface of the microvilli (Steegmaier *et al.*, 1997, J. Cell Sci. 110:687-694, Gonatas *et al.*, 1998, J.Cell Sci. 111:249-260). ESL-1

was identified as ligand for E-selectin in neutrophils with an approximate molecular mass of 150 kD. Immunoprecipitation with anti ESL-1 antibodies showed that a non-defined isoform of this protein could be precipitated from various cells, including some cancerous cell lines (Steegmaier *et al.*, 1995, Nature 373:615-620).

The tissue distribution shows that the tumor- and pre-cancerous lesion-specific isoform of CFR-1 described herein is correlated with cellular activation and proliferation demonstrated by staining with antibody Ki67 (Ramires *et al.*, 1997, J. Pathol. 182:62-67). Variable cellular distribution of a protein is a known phenomenon (Smalheiser, 1996, Mol. Biol. Cell 7:1003-1014). An altered distribution might be caused by a different glycosylation pattern in malignant cells, which may lead to a transport to the plasma membrane. Normal stomach mucosa does not express this receptor in a measurable amount, but *H. pylori* infiltrated epithelia and dysplastic epithelia express this antigen. Both tissues proliferate and may be precursors for gastric carcinoma.

In contrast to the previously described structure of CFR-1, which is found in normal cells, the isoform of CFR-1 described herein is not found on normal cells, but exclusively on proliferating cells associated with malignancies, such as the tumor cells found in the growth and corresponding precursor stages. Thus, the CFR-1 isoform described herein may be used not only in the diagnosis and treatment of gastric carcinomas, but rather also for all epithelial tumors. Besides gastric tumors, the existence of these receptors was proven in cancerous tissue of the following tumors: esophagus, stomach, intestines, rectum, liver, gallbladder, pancreas, lungs, bronchi, breast, cervix, prostate, cardiac, Barrett's, ovary, and/or uterus. An antibody, e.g., a PAM-1 antibody, which binds to the isoform of CFR-1 described herein therefore has a targeted activity on the cancerous or pre-cancerous (and not normal) cells.

Antibodies and Polypeptides

Antibodies play an essential role in maintaining the health of an individual. In particular, antibodies are present in serum and bind to and help eliminate diverse pathogens such as bacteria, viruses, and toxins. Antibodies consist of Y-shaped protein structures built from two heavy chains and two light chains. Each chain has a

modular construction: each light chain consists of two domains, and each heavy chain has at least four domains. The antigen binding site is fashioned by one domain from the heavy chain (V_H domain) and one domain from the light chain (V_L domain). Indeed, small antigen binding fragments can be prepared by linking these two domains, either associated non-covalently, or covalently via disulphide bonds or a peptide linker. The antigen binding domains are more variable in amino acid sequence than the other domains of the antibody, and are therefore termed variable (V) domains, in contrast to the constant (C) domains. The constant domains of the antibody are responsible for triggering antibody effector mechanisms, such as complement lysis and cell-mediated killing.

Antibodies are made by B-lymphocytes in a process involving gene rearrangement. During the development of these cells, the genes encoding the variable domains are assembled from genetic elements. In the case of the V_H domains there are three elements, the un-rearranged V_H gene, D segment, and J_H segment. In the case of the V_L domains, there are two elements, the un-rearranged V_L (V Lambda or V Kappa) gene and the J_L (J Lambda or J Kappa) segment. Random combination of these gene segments and random combination of the rearranged V_H and V_L domains generate a large repertoire of antibodies, capable of binding to a large number of equally diverse antigens. Further, the V_H and V_L regions each have three Complement Determining Regions (CDR) and four framework regions (FR). The FRs are the backbone of the antibody and the CDRs are the parts of the antibody that bind the antigen. One skilled in the art can determine the FR and CDR regions of an antibody by comparing the amino acid sequence of a number of antibodies raised in the same species (see, e.g., Altschul et al., Nucleic Acids Res. 25:3389-3402, 1997; and Kabat et al., Sequences of Proteins of Immunological Interest, 5th edition, NIH Publication No. 91-3242, U.S. Department of Health and Human Services, 1991).

Production of Neoplasm-Specific Polypeptides

A PAM-1 antibody or a fragment thereof may be produced by expression in a hybridoma or recombinantely in a host cell such as *E. coli* or yeast, e.g., *S. cerevisiae*, or a mammalian cell line. Functional fragments of polypeptides may also be

generated, for example, by direct synthesis using recombinant methods. These methods are standard in the art. For example, a nucleic acid sequence may be amplified using the polymerase chain reaction (PCR). The PCR technique is known in the art and is described, for example in U.S. Patent No. 4,683,195. Using standard methods, and as described herein, the sequence of a monoclonal antibody expressed by a hybridoma or trioma may be obtained and functional fragments of the antibody may be amplified. For example, whole RNA may be isolated from a hybridoma expressing a tumor-specific monoclonal antibody. cDNA may then be generated from the RNA using reverse transcriptase and the cDNAs which contain the functional fragments of the variable regions of the heavy and light chains may be amplified using PCR. The PCR products may then be purified and cloned into expression vectors, e.g., plasmid or viral vectors. Many standard vectors are available and the selection of the appropriate vector will depend on, for example, the size of the DNA inserted into the vector and the host cell to be transfected with the vector.

The nucleic acid molecules identified using the methods of the invention may be expressed in a variety of standard vectors and host cells. Any promoter that is active in the host cell may be used to express a nucleic acid molecule. Nonetheless, for expression of an antibody or a fragment of an antibody in a mammalian cell, use of an immunoglobulin gene promoter is desirable. Methods of introducing a vector into a host cell are standard in the art and include, electroporation, use of synthetic lipid polymers, e.g., Lipofectin™, use of calcium chloride, and use of DEAE Dextran. Such methods are also described in, for example, Ausubel *et al., Current Protocols in Molecular Biology*, Wiley Interscience, New York, 2001; and Sambrook et al., *Molecular Cloning: A Laboratory Manual*, 3rd edition, Cold Spring Harbor Laboratory Press, N.Y., 2001.

In addition, the purified antigen recognized by an antibody (e.g., the novel isoform of CFR-1 recognized by human PAM-1 antibody 103/51), or a fragment thereof, may be used to generate additional antibodies that specifically recognize the same antigen. Such methods are standard in the art and generally involve immunizing a mammal, such as a mouse, rat, rabbit, goat, or horse, with the purified antigen to illicit an immune response against the antigen in the mammal. Antibodies produced

by the mammal in response to this immunization may either be purified from blood obtained from the mammal and characterized for binding specificity and function, or antibody producing lymphocytes or splenocytes may be obtained from the mammal and used to generate antibody-producing hybridoma cell lines. The antibodies produced by these hybridoma cell lines are then characterized for binding specificity and function using standard assays as described herein.

Isolation of Amino Acid Variants of a PAM-1 Antibody

Amino acid sequence variants of a PAM-1 antibody can be prepared by introducing appropriate nucleotide changes into the DNA encoding the antibody, or by *in vitro* synthesis of the desired polypeptide. Such variants include, for example, deletion, insertion, or substitution of, residues within the amino acid sequence of a PAM-1 antibody. Any combination of deletion, insertion, and substitution can be made to arrive at the final construct, provided that the final construct possesses the desired characteristics, e.g., the ability to induce apoptosis of a neoplastic cell, but not a non-neoplastic cell, or the ability to inhibit the proliferation of a cell. The amino acid changes also may alter post-translational processes of an antibody, such as changing the number or position of glycosylation sites, altering the membrane anchoring characteristics, or modifying its susceptibility to proteolytic cleavage.

In designing amino acid sequence variants of a polypeptide, such as an antibody, the location of the mutation site and the nature of the mutation will depend on characteristic(s) to be modified. The sites for mutation can be modified individually or in series, e.g., by substituting first with conservative amino acid choices and then with more radical selections depending upon the results achieved, or deleting the target residue.

A useful method for identification of specific residues or regions for mutagenesis in a polypeptide is called "alanine scanning mutagenesis" and is described, for example, by Cunningham and Wells (Science 244:1081-1085, 1989). Here, a residue or group of target residues are identified (e.g., charged residues such as arg, asp, his, lys, and glu) and replaced by a neutral or negatively charged amino acid (most desirably alanine or polyalanine) to affect the interaction of the amino acids

with the surrounding aqueous environment in or outside the cell. The domains demonstrating functional sensitivity to the substitutions then are refined by introducing further or other variants at or for the sites of substitution. Thus, while the site for introducing an amino acid sequence variation is predetermined, the nature of the mutation need not be predetermined. For instance, to optimize the performance of a mutation at a given site, alanine scanning or random mutagenesis may be conducted at the target codon or region and the expressed variants are screened for, e.g., the ability to induce apoptosis of a neoplastic cell and not a non-neoplastic cell, or to inhibit the proliferation of a neoplastic cell and not a non-neoplastic cell.

The sites of greatest interest for substitutional mutagenesis include sites identified as affecting the biological activity of a polypeptide. These sites, especially those falling within a sequence of at least three other identically conserved sites, may be substituted in a relatively conservative manner. For instance, ala may be substituted with val, leu, or ile; arg may be substituted with lys, gln, or asn; asn may be substituted with gln, his, lys, or arg; asp may be substituted with glu; cys may be substituted with ser; gln may be substituted with asn; glu may be substituted with asp; gly may be substituted with pro; his may be substituted with asn, gln, lys, or arg; ile may be substituted with leu, val, met, ala, or phe; leu may be substituted with ile, val, met, ala, or phe; lys may be substituted with arg, gln, or asn; met may be substituted with leu, phe, or ile; phe may be substituted with leu, val, ile, or ala; pro may be substituted with gly; ser may be substituted with thr; thr may be substituted with ser; trp may be substituted with tyr; tyr may be substituted with trp, phe, thr, or ser; and val may be substituted with ile, leu, met, or phe.

Conjugation of a Polypeptide with a Detectable Agent

If desired, a PAM-1 antibody, a fragment thereof, or the novel CFR-1 isoform described herein may be linked to a detectable agent to facilitate the purification of the polypeptide as well as the diagnosis, monitoring, or treatment of a neoplasm or a pre-cancerous lesion in a mammal in need thereof. The selection of suitable detectable agent will depend on the intended use of the polypeptide and will be apparent to those of ordinary skill in the art. Detectable agents according to the invention include, for

example, protein purification tags, cytotoxins, enzymes, paramagnetic labels, enzyme substrates, co-factors, enzyme inhibitors, dyes, radionuclides, chemiluminescent labels, fluorescent markers, growth inhibitors, and biotin.

A protein purification tag may be conjugated to a PAM-1 antibody, fragment thereof, or the novel CFR-1 isoform described herein to facilitate isolation of the polypeptide. Examples of tags that can be used include His-tags, HA-tags, FLAG®-tags, and c-Myc tags. An enzymatic or a chemical cleavage site may be engineered between the polypeptide and the tag moiety so that the tag can be removed following purification. Suitable toxins include diphtheria toxin, Pseudomonas exotoxin A, ricin, and cholera toxin. Examples of suitable enzyme labels include malate hydrogenase, staphylococcal nuclease, delta-5-steroid isomerase, alcohol dehydrogenase, alpha-glycerol phosphate dehydrogenase, triose phosphate isomerase, peroxidase, alkaline phosphatase, asparaginase, glucose oxidase, beta-galactosidase, ribonuclease, urease, catalase, glucose-6-phosphate dehydrogenase, glucoamylase, and acetylcholinesterase. Examples of suitable radioisotopic labels include ^{3}H, ^{125}I, ^{131}I, ^{32}P, ^{35}S, and ^{14}C. Desirably, the radioisotope will emit in the 10-5,000 kev range, more desirably 100-500 kev. Paramagnetic isotopes may also be conjugated to the polypeptide and used *in vivo* for the diagnosis and treatment of cancer. The use of such conjugated polypeptides may be for *in vivo* nuclear magnetic resonance imaging. Such methods are known in the art (see, for example, Schaefer et al., JACC 14:472-480, 1989; Shreve et al., Magn. Reson. Med. 3:336-340, 1986; Wolf, Physiol. Chem. Phys. Med. NMR 16:93-95, 1984; Wesbey et al., Physiol. Chem. Phys. Med. NMR 16:145-155, 1984; and Runge et al., Invest. Radiol. 19:408-415, 1984). Alternatively, a radiolabeled polypeptide may also be used in radioimmunoguided surgery (RIGS), which involves the surgical removal of any tissue the labeled antibody binds to. Thus, the labeled polypeptide guides the surgeon towards neoplastic tissue by distinguishing it from non-neoplastic tissue. Radiolabels useful for tumor imaging are preferably short-lived radioisotopes. Various radioactive metals with half-lives ranging from 1 hour to 11.4 days are available for conjugation to antibodies, such as scandium-47 (3.4 days), gallium-67 (2.8 days), gallium-68 (68 minutes), technetium-99m (6 hours), indium-111 (3.2 days), and radium-223 (11.4 days), of which gallium-67, technetium-

99m, and indium-111 are preferable for gamma camera imaging, gallium-68 is preferable for positron emission tomography, and scandium-47 and radium-223 (and other alpha-emitting radionuclides) are preferable for tumor therapy.

Examples of suitable fluorescent markers include fluorescein, isothiocyalate, rhodamine, phycoerythrin, phycocyanin, allophycocyanin, ophthaldehyde, and fluorescamine. Examples of chemiluminescent markers include a luminal label, isoluminal label, aromatic acridinium ester label, imidazole label, acridinium salt label, oxalate ester label, luciferin label, luciferase label, and aequorin label. Those of ordinary skill in the art would know of other suitable labels, which may be employed in accordance with the present invention. Conjugation of these detectable agents to a polypeptide described herein, such as PAM-1 antibodies, fragments thereof, and the novel isoform of CFR-1, can be accomplished using standard techniques known in the art. Typical antibody conjugation techniques are described by Kennedy et al. (*Clin. Chim. Acta* 70, 1-31, 1976) and Schurs et al. (*Clin. Chim. Acta* 81, 1-40 , 1977) and include, for example, the glutaraldehyde method, the periodate method, the dimaleimide method, the m-maleimidobenzyl-N-hydroxy-succinimide ester method. Antibodies may be radiolabeled by any of several techniques known to the art, described, for example, in U.S. patent No. 4,444,744. All of these methods are incorporated by reference herein.

In all methods of treatment of the present invention, it is understood that mixtures of different or the same labeled antibodies specific to different antigens or different epitopes of the same antigen associated with the same or different tumor or tumor cell types may be used. Such a combination may enhance detection, localization, and/or therapy in certain cases, and can also increase the range of a broad screen for more than one neoplasm or type of neoplasm.

Polypeptides Conjugated to Anti-Tumor Agents

Although a PAM-1 antibody or fragment thereof can induce apoptosis of neoplastic cells, inhibit cellular proliferation of neoplastic cells, or both, the polypeptide may in addition be conjugated to an agent that kills neoplastic cells or that

inhibits their proliferation. The targeting ability of the PAM-1 antibody or fragment thereof, results in the delivery of the cytotoxic or anti-proliferative agent to the tumor to enhance the destruction of the tumor. The polypeptide therefore may be used for the treatment and prevention of a neoplasm or pre-cancerous lesion in a mammal, such as a human patient. The cytotoxic agent linked to the polypeptide may be any agent that destroys or damages a tumor cell or tumor to which the polypeptide has bound. Examples of such agents include chemotherapeutic agents or radioisotopes, enzymes which activate a pro-drug, or a cytokine.

Suitable chemotherapeutic agents are known to those skilled in the art and include, for example, taxol, mithramycin, deoxyco-formycin, mitomycin-C, L-asparaginase, interferons (especially IFN-alpha), etoposide, teniposide, anthracyclines (e.g., daunomycin and doxorubicin), methotrexate, vindesine, neocarzinostatin, cis-platinum, chlorambucil, cytosine arabinoside, 5-fluorouridine, melphalan, ricin, and calicheamicin. The chemotherapeutic agents may be conjugated to the antibody using conventional methods known in the art.

Suitable radioisotopes for use as cytotoxic agents are also known to those skilled in the art and include, for example, ^{131}I, or an astatine such as ^{211}At. These isotopes may be attached to the polypeptide, either covalently or non-covalently, using conventional techniques known in the art.

Alternatively, the cytotoxic agent may also be an enzyme, which activates a pro-drug. This allows the conversion of an inactive pro-drug to its active, cytotoxic form at the tumor site and is called "antibody-directed enzyme pro-drug therapy" (ADEPT). Thus, the polypeptide-enzyme conjugate may be administered to the patient and allowed to localize in the region of the tumor to be treated. The pro-drug is then administered to the patient such that conversion to the cytotoxic drug is localized in the region of the tumor to be treated under the influence of the localized enzyme. An exemplary enzyme is bacterial carboxypeptidase G2 (CPG2) the use of which is described in, for example, WO 88/07378. The polypeptide-enzyme conjugate may, if desired, be modified in accordance with the teaching of WO 89/00427, such as to accelerate its clearance from areas of the body that are not in the vicinity of a neoplasm. The polypeptide-enzyme conjugate may also be used in

accordance with WO 89/00427, for example, by providing an additional component, which inactivates the enzyme in areas of the body that are not in the vicinity of the tumor.

As another alternative, the cytotoxic agent conjugated to a PAM-1 antibody or fragment thereof may also be a cytokine such as interleukin-2 (IL-2), interleukin-4 (IL-4), or tumor necrosis factor alpha (TNF-alpha). The polypeptide targets the cytokine to the tumor so that the cytokine mediates damage to or destruction of the tumor without affecting other tissues. The cytokine may be fused to the polypeptide at the DNA level using conventional recombinant DNA techniques.

In addition, any inhibitor of cell proliferation. e.g., genistein, tamoxifen, or cyclophosphamide, may be conjugated with a polypeptide described herein.

Dosage

With respect to the therapeutic methods of the invention, it is not intended that the administration of a polypeptide of the invention to a patient be limited to a particular mode of administration, dosage, or frequency of dosing; the present invention contemplates all modes of administration, including intramuscular, intravenous, intraperitoneal, intravesicular, intraarticular, intralesional, subcutaneous, or any other route sufficient to provide a dose adequate to decrease the number of neoplastic cells by inducing apoptosis of neoplastic cells, by inhibiting proliferation of tumor cells, or both. The compound(s) may be administered to the patient in a single dose or in multiple doses. When multiple doses are administered, the doses may be separated from one another by, for example, one day, two days, one week, two weeks, or one month. For example, the polypeptide (e.g., a PAM-1 antibody, fragment thereof, the novel CFR-1 isoform described herein), or a vector including a nucleic acid molecule that encodes this novel isoform of CFR-1, may be administered once a week for, e.g., 2, 3, 4, 5, 6, 7, 8, 10, 15, 20, or more weeks. It is to be understood that, for any particular subject, specific dosage regimes should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions. The precise dose will vary dependent on the polypeptide or vector used, the density, on the tumor

surface, of the ligand to which the polypeptide binds, and the rate of clearance of the polypeptide. For example, the dosage of a PAM-1 antibody or novel isoform of CFR-1 described herein can be increased if the lower dose does not provide sufficient anti-neoplastic activity. Conversely, the dosage a PAM-1 antibody or novel isoform of CFR-1 described herein can be decreased if the neoplasm is cleared from the patient.

While the attending physician ultimately will decide the appropriate amount and dosage regimen, a therapeutically effective amount of a polypeptide, such as a monoclonal antibody or a fragment thereof, may be, for example, in the range of about 0.1 mg to 50 mg/kg body weight/day or 0.70 mg to 350 mg/kg body weight/week. Desirably a therapeutically effective amount is in the range of about 0.50 mg to 20.0 mg/kg, and more desirably in the range of about 0.50 mg to 15.0 mg/kg, for example, about 0.2, 0.3, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 7.0, 8.0, 8.5, 9.0, 10.0, 11.0, 12.0, 13.0, 14.0, or 15.0 mg/kg body weight administered daily, every other day, or twice a week.

For instance, a suitable dose is an amount of the polypeptide that, when administered as described above, is capable of inducing apoptosis, and is at least 20% above the basal (i.e., untreated) level. In general, an appropriate dosage and treatment regimen provides the active compound(s) in an amount sufficient to provide therapeutic and/or prophylactic benefit. Such a response can be monitored by establishing an improved clinical outcome (e.g., more frequent remissions, complete or partial, or longer disease-free survival) in treated patients as compared to non-treated patients. According to this invention, the administration of the polypeptide can induce neoplastic cell apoptosis by at least 20%, 40%, 50%, or 75% above that of an untreated control as measured by any standard assay known in the art. More desirably, apoptosis is induced by 80%, 90%, 95%, or even 100% above that of an untreated control. Alternatively, the administration of the polypeptide can inhibit neoplastic cell proliferation by at least 20%, 40%, 50%, or 75% below that of an untreated control as measured by any standard assay known in the art. More desirably, proliferation is inhibited by 80%, 90%, 95%, or even 100% below that of an untreated control. Most desirably, the polypeptide can simultaneously inhibit proliferation and induce apoptosis of neoplastic cells relative to untreated control

cells. Such responses can be monitored by any standard technique known in the art, including those described herein. In general, for pharmaceutical compositions, the amount of antibody present in a dose ranges from about 25 μg to 5 mg per kg of host. Suitable dose sizes will vary with the size of the patient, but will typically range from about 0.1 mL to about 5 mL.

In addition, a DNA vaccine including a plasmid vector or a viral vector which includes a nucleotide sequence encoding SEQ ID NO:6, or a fragment thereof, may be used to induce a tumor-specific immune response in a patient. This immune response, for example, results in the formation of antibodies that specifically bind to a neoplasm or a pre-cancerous lesion. In general, an effective dose range of about 1 ng to 5 mg, 100 ng to 2.5 mg, 1 μg to 750 μg, and preferably about 10 μg to 300 μg of DNA is administered directly into muscle tissue. Subcutaneous injection, intradermal introduction, impression through the skin, and other modes of administration such as intraperitoneal, intravenous, or inhalation delivery are also suitable. In addition, booster vaccinations may be provided.

Formulation of Pharmaceutical Compositions

A PAM-1 antibody, fragment thereof, or the novel CFR-1 isoform described therein may be administered by any suitable means that results in a concentration having anti-neoplastic properties upon reaching the target region. The polypeptide may be contained in any appropriate amount in any suitable carrier substance, and is generally present in an amount of 1-95% by weight of the total weight of the composition. The composition may be provided in a dosage form that is suitable for parenteral (e.g., subcutaneous, intravenous, intramuscular, or intraperitoneal) administration route. The pharmaceutical compositions may be formulated according to conventional pharmaceutical practice (see, e.g., Remington: The Science and Practice of Pharmacy (20th ed.), ed. A.R. Gennaro, Lippincott, Williams & Wilkins, 2000 and Encyclopedia of Pharmaceutical Technology, eds. J. Swarbrick and J. C. Boylan, 1988-1999, Marcel Dekker, New York).

The pharmaceutical composition may be administered parenterally by injection, infusion or implantation (subcutaneous, intravenous, intramuscular,

intraperitoneal, or the like) in dosage forms, formulations, or via suitable delivery devices or implants containing conventional, non-toxic pharmaceutically acceptable carriers and adjuvants. If the neoplastic cells are in direct contact with the blood (e.g., leukemias), or if the tumor is only accessible by the bloodstream then the intravenous (I.V.) route may be used. In cases in which tumors grow in confined spaces such as the pleural cavity or the peritoneal cavity, the polypeptide may be directly administered into the cavity rather than into the blood stream. The formulation and preparation of such compositions are well known to those skilled in the art of pharmaceutical formulation. Formulations can be found, for example, in Remington (The Science and Practice of Pharmacy (20th ed.), ed. A.R. Gennaro, Lippincott, Williams & Wilkins, 2000 and Encyclopedia of Pharmaceutical Technology, eds. J. Swarbrick and J. C. Boylan, 1988-1999, Marcel Dekker, New York).

Diagnosis and Monitoring Cancer Progression

As discussed above, aspects of the present invention are directed to methods of detecting or diagnosing a neoplasm in a mammal, preferably a human patient. Typically, any neoplasm which is specifically bound by a PAM-1 antibody, or a fragment thereof, may be detected either *in vitro* or *in vivo* according to the methods of the present invention. Such a neoplasm may be one that expressed the isoform of CFR-1 that is bound by a PAM-1 antibody. Furthermore, a cell which expressed the isoform of CFR-1 described herein is likely to be a neoplastic cell or a cell in a pre-cancerous lesion. Thus, the isoform of CFR-1 recognized by a PAM-1 antibody may be used as a marker to detect whether a patient has neoplasm or is likely to develop a neoplasm.

PAM-1 antibodies and fragments thereof are particularly useful because they specifically bind neoplastic, cells in pre-cancerous lesions, as well as proliferating malignant cells, and not normal cells and normal proliferating cells or tissue. Accordingly, such polypeptides can bind to neoplastic cells within the tumor, but not the normal surrounding tissue, thus allowing the detection, the treatment, or both, of a neoplasm in a mammal. For instance, PAM-1 antibodies and fragments thereof may be used to determine if a biopsy removed the entire tumor by verifying that no cells

bound by the polypeptide remain in the patient or, by verifying that tumor removed from the patient is entirely surrounded by cells that are not bound by the polypeptide.

It is understood that to improve the sensitivity of detection, multiple neoplastic markers may be assayed within a given sample or individual. Thus, polypeptides such as antibodies or functional fragments specific for different antigens may be combined within a single assay, or in multiple assays. Further, multiple primers or probes specific to neoplasms may be used concurrently. The selection of markers may be based on routine experiments to determine combinations that results in optimal sensitivity.

In Vitro Detection of a Neoplasm

In general, the diagnosis of a neoplasm in a mammal involves obtaining a biological sample from the mammal (e.g., human patient), contacting such sample with a PAM-1 antibody or fragment thereof, detecting, in the test sample, the level of reactivity or binding of the polypeptide to neoplastic cells relative to a control sample, which corresponds to non-neoplastic cells derived from healthy tissue from the mammal in which the cancer is being diagnosed or from another patient known not to have a neoplasm. Thus, the novel isoform of CFR-1 described herein, as well as the PAM-1 antibodies of the invention are particularly useful for the detection of early stage tumors or metastases, which are otherwise undetectable. Accordingly, in addition to diagnosing a neoplasm in a patient, the methods of this invention may also be used to monitor progression of a neoplasm in a mammal. The polypeptides described herein therefore may be used as markers for the progression of a neoplasm. For this purpose, the assays described below, which are used for the diagnosis of a neoplasm, may be performed over time, and the change in the level of reactive polypeptide(s) evaluated. For example, the assays may be performed every 24-72 hours for a period of 6 months to 1 year, and thereafter performed as needed. In general, a neoplasm is progressing in those patients in whom the level of bound PAM-1 antibody detected increases over time or where the expression of the isoform of CFR-1 described herein increases over time. In contrast, the neoplasm is not progressing when the level of bound PAM-1 antibody either remains constant or

decreases with time or where the expression of the isoform of CFR-1 described herein descreases over time. Alternatively, as is noted above, the CFR-1 isoform described herein and the PAM-1 antibodies may be used to determine the presence of tumor cells in the mammal following tumor resection by surgical intervention to determine whether the tumor has been completely removed from the mammal.

Desirably, the polypeptide is linked to a detectable agent, which facilitates detection, or measurement of polypeptide reactivity. The biological sample is any biological material which may contain neoplastic or proliferating cells and includes, for example, blood, saliva, tissue, serum, mucus, sputum, urine, or tears. The biological sample may also be a tissue section, which may be fixed tissue, fresh tissue, or frozen tissue. A neoplasm is detected or diagnosed in the mammal from which the sample was obtained if there is an increase in the level of reactivity of a PAM-1 antibody or fragment thereof with the biological sample, or increased expression of the isoform of CFR-1 described herein over the control sample. Such increase is at least 10%, 20%, 30%, 40%, 50%, or more than 50% over control levels. The level of binding or reactivity can be determined by any method known in the art and is described in further detail below.

In Vitro Diagnostic Assays

The diagnosis of neoplasms or a pre-cancerous lesion using a polypeptide of the invention may be performed by any method known to those of ordinary skill in the art for using a binding agent to detect polypeptide markers, e.g., the isoform of CFR-1 recognized by a PAM-1 antibody, in a sample. See, e.g., Harlow and Lane, Using Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, N.Y., 1999. For example, the polypeptide may be used for enzyme-linked immunosorbent assay (ELISA), Western blotting, or *in situ* detection of tumor cells in a tissue sample. For instance, the ELISA assay typically involves the use of a polypeptide, such as an antibody, immobilized on a solid support to bind to the tumor cells in the biological sample. The bound tumor cell may then be detected using a detection reagent that contains a reporter group and that specifically binds to the antibody/tumor cell complex. Such detection reagents include, for example, any binding agent that

specifically binds to a PAM-1 antibody or fragment thereof, such as an anti-immunoglobulin, protein G, protein A, or a lectin. Alternatively, a competitive assay may be utilized, in which the polypeptide is a PAM-1 antibody and in which the antigens, to which the antibody is specific to is labeled with a reporter group and allowed to bind to the immobilized antibody after incubation of the antibody with the biological sample. The extent to which components of the sample inhibit the binding of the labeled antigens to the antibody is indicative of the reactivity of the sample with the immobilized antibody. Diagnosis of a neoplasm in a patient, or the identification of a pre-cancerous lesion in a patient, may also be determined by a two-antibody sandwich assay. This assay may be performed by first contacting an antibody that has been immobilized on a solid support, commonly the well of a microtiter plate, with the sample, such that polypeptides within the sample are allowed to bind to the immobilized antibody. Unbound sample is then removed from the immobilized polypeptide-antibody complexes and a detection reagent (preferably a second antibody capable of binding to a different site on the polypeptide) containing a reporter group is added. The amount of detection reagent that remains bound to the solid support is then determined using a method appropriate for the specific reporter group. For example, to determine the presence or absence of a neoplasm, such as a stomach adenocarcinoma, the signal detected from the reporter group that remains bound to the solid support is generally compared to a signal that corresponds to a predetermined cut-off value. The cut-off value for the detection of a neoplasm is the average mean signal obtained when the antibody is incubated with samples from patients without a neoplasm.

The method employed for detecting the reporter group depends upon the nature of the reporter group. For radioactive groups, scintillation counting or autoradiographic methods may be used. Spectroscopic methods may be used to detect dyes, luminescent groups and fluorescent groups. Biotin may be detected using avidin, coupled to a different reporter group (commonly a radioactive or fluorescent group or an enzyme). Enzyme reporter groups may generally be detected by the addition of substrate (generally for a defined period of time), followed by spectroscopic or other analysis of the reaction products.

PAM-1 antibodies and fragments thereof may also be employed histologically for *in situ* detection or quantitative determination of tumor cells, for example, by immunofluorescence or immunoelectron microscopy. *In situ* detection or determination may be accomplished by removing a tissue specimen from a patient and allowing a labeled antibody to bind to any tumor cell in the specimen. Using such a procedure not only allows the detection of neoplastic or a pre-cancerous lesion in a sample, but also allows for the determination of their spatial distribution. As another example, the biological sample can be a smear of biological material containing neoplastic cells on a slide, and the detection of neoplastic cells in the biological material is achieved by examining the smear with a microscope or by fluocytometry.

In Vivo detection of a Neoplasm

Alternatively, A PAM-1 antibody may also be used *in vivo* for detecting and localizing a neoplasm or pre-cancerous lesion. Such a method may involve injecting a mammal, desirably a human subject, parenterally with a PAM-1 antibody or fragment thereof, which has been labeled with a detectable agent, and is described, for instance, in U.S. Patent No. 4,444,744. For example, the antibody or antibody fragment can be radiolabeled with a pharmacologically inert radioisotope and administered to the patient. The activity of the radioisotope can be detected in the mammal using a photoscanning device, and an increase in activity relative to a control reflects the detection and localization of a neoplasm.

Treatment

In addition to the diagnosis and monitoring of neoplasms in mammals, the present invention also features methods for treating neoplasms in a mammal, desirably a human patient. The method generally involves the administration of a biologically effective amount of a polypetide, e.g., the isoform of CFR-1 described herein, or a PAM-1 antibody or fragment thereof. The polypeptide is typically administered to the mammal by means of injection using any routes of administration such as by intrathecal, subcutaneous, submucosal, or intracavitary injection as well as for intravenous or intraarterial injection. Thus, the polypeptide may be injected

systemically, for example, by the intravenous injection of the polypeptide into the patient's bloodstream or alternatively, the polypeptide can be directly injected at the site of the neoplasm or at a location in proximity to the neoplastic cells.

For example, purified polypeptides corresponding to all or a part of the CFR-1 isoform described herein may be used to reinforce and amplify antibody formation and, therefore, may be used to induce elevated apoptosis of the tumor cells or for a complement-mediated lysis. Further, given CFR-1's likely function in nutrient uptake, the cells expressing this tumor-specific isoform of CFR-1 would "starve," since blocking of the receptor leads to growth arrest.

In view of its tissue distribution, the isoform of CRF-1 described herein is particularly suitable for treating the following tumors and pre-cancerous lesions: dysplasia of the gastric mucosa, interstitial metaplasia of the stomach, inflammation of the gastric mucosa which is associated with the bacteria *Helicobacter pylori*, tubular and tubulovillous adenomas of the stomach, tubular adenoma of the colon, villous adenoma of the colon, dysplasia in ulcerative colitis, Barrett's dysplasia, Barrett's metaplasia of the esophagus, cervical intraepithelial neoplasia I, cervical intraepithelial neoplasia II, cervical intraepithelial neoplasia III, squamous epithelial metaplasia, squamous epithelial dysplasia of the bronchus, low grade and high grade prostate intraepithelial neoplasia (PIN), breast ductal carcinoma in situ (D-CIS), breast lobular carcinoma in situ (L-CIS), Barrett's tumors, and tumors of the esophagus, stomach, intestine, rectum, liver, gallbladder, pancreas, lungs, bronchi, breast, cervix, prostate, heart, ovary, and uterus.

Furthermore, PAM-1 antibodies and fragments thereof may also be used in methods to treat neoplasms or kill cells of a pre-cancerous lesion. As discussed above, binding of a PAM-1 antibody or fragment thereof to a neoplastic or a cell of a pre-cancerous lesion results in an induction in apoptosis, a reduction in cellular proliferation, or both relative to the control sample. Alternatively, the antibodies may also activate the complement pathway, which ultimately causes holes to be punctured into the cellular membrane, resulting in cell death.

If desired, the PAM-1 antibody or fragment thereof may also be conjugated to drugs or toxins as described above. Once attached to the cell surface, the conjugate

may be engulfed into the cell cytoplasm where cell enzymes cleave, and, thus, activate or free the drugs or toxins from the conjugate. Once released, the drugs or toxins damage the cell and irreversibly induce cell death. With respect to radiolabeled antibodies, binding to neoplastic cells or cells of a pre-cancerous lesion and the resulting emission of radiation, at a short distance from the cell DNA, produces damage to the latter thus inducing cell death in the next replication round. For example, after a neoplasm has been detected and localized in a subject, a higher dose of labeled antibody, generally from 25 to 250 mCi for ^{131}I, and preferably from 50 nCi to 150 mCi per dose, based on a 70 kg patient weight, is injected. Injection may be intravenous, intraarterial, intralymphatic, intrathecal, or intracavitary, and may be repeated more than once. It may be advantageous for some therapies to administer multiple, divided doses of radiolabeled polypeptides or polypeptide mixtures, e.g., in the range of 20-120 mCi (70 kg patient), thus providing higher cell-killing doses to the neoplasm usually without effecting a proportional increase in radiation of normal tissues

Therapy using labeled polypeptides is advantageously used as a primary therapeutic treatment, but may also be used in combination with other anti-neoplastic therapies, e.g., radiation and chemotherapy, and as an adjunct to surgery. The administration of such conjugated polypeptides is particularly useful in the case where small metastases cannot be surgically removed.

Combination of a Polypeptide with other Anti-Neoplastic Therapies

Chemotherapeutic agents and/or radiation and/or surgical removal of the neoplasm can optionally be combined with any of the methods of the present invention. Classes of compounds that can be used as the chemotherapeutic agent include: alkylating agents, antimetabolites, natural products and their derivatives, hormones and steroids (including synthetic analogs), and synthetics. Examples of alkylating agents (e.g., nitrogen mustards, ethylenimine derivatives, alkyl sulfonates, nitrosoureas and triazenes) include Uracil mustard, Chlormethine, Cyclophosphamide (Cytoxan®), Ifosfamide, Melphalan, Chlorambucil, Pipobroman, Triethylene-melamine, Triethylenethiophosphoramine, Busulfan, Carmustine, Lomustine,

Streptozocin, Dacarbazine, and Temozolomide. Antimetabolites (including folic acid antagonists, pyrimidine analogs, purine analogs and adenosine deaminase inhibitors) may include, for example, Methotrexate, 5-Fluorouracil, Floxuridine, Cytarabine, 6-Mercaptopurine, 6-Thioguanine, Fludarabine phosphate, Pentostatine, and Gemcitabine. Natural products and their derivatives (including vinca alkaloids, antitumor antibiotics, enzymes, lymphokines and epipodophyllotoxins) may also be used and include, for example, Vinblastine, Vincristine, Vindesine, Bleomycin, Dactinomycin, Daunorubicin, Doxorubicin, Epirubicin, Idarubicin, paclitaxel (paclitaxel is commercially available as Taxol®), Mithramycin, Deoxyco-formycin, Mitomycin-C, L-Asparaginase, Interferons (especially IFN-alpha), Etoposide, and Teniposide. Hormones and steroids (including synthetic analogs) include, for example, 17-alpha-Ethinylestradiol, Diethylstilbestrol, Testosterone, Prednisone, Fluoxymesterone, Dromostanolone propionate, Testolactone, Megestrolacetate, Tamoxifen, Methylprednisolone, Methyltestosterone, Prednisolone, Triamcinolone, Chlorotrianisene, Hydroxyprogesterone, Aminoglutethimide, Estramustine, Medroxyprogesteroneacetate, Leuprolide, Flutamide, Toremifene, or Zoladex. Exemplary synthetics (including inorganic complexes such as platinum coordination complexes) include Cisplatin, Carboplatin, Hydroxyurea, Amsacrine, Procarbazine, Mitotane, Mitoxantrone, Levamisole, and Hexamethylmelamine.

Methods and dosages for the safe and effective administration of most of these chemotherapeutic agents are known to those skilled in the art. In addition, their administration is described in the standard literature. For example, the administration of many of the chemotherapeutic agents is described in the "Physicians' Desk Reference" (PDR), e.g., 1996 edition (Medical Economics Company, Montvale, N.J. 07645-1742, USA), the disclosure of which is incorporated herein by reference.

Identification of therapeutic compounds

The novel isoform of CFR-1 described herein, or fragments thereof, may be used to produce an anti-neoplastic or anti-proliferative agent, in which compounds that are potentially effective against tumors are assayed for their ability to specifically bind to the novel CFR-1 isoform described herein. Upon a positive result, i.e., upon

the occurrence of binding, this compound may be assayed for its ability to induce apoptosis in neoplastic cells or cells of a pre-cancerous lesion or its ability to alter the rate of proliferation of such cells using the methods described herein. Once such a compound has been characterized, it may be included in a pharmaceutical composition for the treatment of a neoplastic disease.

Test extracts and compounds

In general, compounds that alter a biological activity of the novel isoform of CFR-1 described herein are identified from large libraries of both natural products, synthetic (or semi-synthetic) extracts or chemical libraries, according to methods known in the art. For example, such compounds may be human antibodies, murine antibodies, humanized antibodies of any arbitrary species, as well as antibody fragments such as Fab and $F(ab)_2$ and/or Fab' fragments obtained by proteolytic cleavage of antibodies. These also include single strand antibodies and/or tetrameric and/or dimeric antibody forms and/or bispecific antibodies.

Those skilled in the art will understand that the precise source of test extracts or compounds is not critical to the screening procedure(s) of the invention. Accordingly, virtually any number of chemical extracts or compounds can be screened using the exemplary methods described herein. Examples of such extracts or compounds include, but are not limited to, plant-, fungal-, prokaryotic- or animal-based extracts, fermentation broths, and synthetic compounds, as well as modifications of existing compounds. Numerous methods are also available for generating random or directed synthesis (e.g., semi-synthesis or total synthesis) of any number of chemical compounds, including, but not limited to, saccharide-, lipid-, peptide-, and nucleic acid-based compounds. Synthetic compound libraries are commercially available from, for example, Brandon Associates (Merrimack, NH) and Aldrich Chemical (Milwaukee, WI).

Alternatively, libraries of natural compounds in the form of bacterial, fungal, plant, and animal extracts are commercially available from a number of sources, including, but not limited to, Biotics (Sussex, UK), Xenova (Slough, UK), Harbor Branch Oceangraphics Institute (Ft. Pierce, FL), and PharmaMar, U.S.A. (Cambridge,

MA). In addition, natural and synthetically produced libraries are produced, if desired, according to methods known in the art (e.g., by combinatorial chemistry methods or standard extraction and fractionation methods). Furthermore, if desired, any library or compound may be readily modified using standard chemical, physical, or biochemical methods.

In addition, those skilled in the art readily understand that methods for dereplication (e.g., taxonomic dereplication, biological dereplication, and chemical dereplication, or any combination thereof) or the elimination of replicates or repeats of materials already known for their effects on CFR-1 should be employed whenever possible.

When a crude extract is found to alter a biological activity of the novel CFR-1 isoform described herein, further fractionation of the positive lead extract is necessary to isolate chemical constituents responsible for the observed effect. Thus, the goal of the extraction, fractionation, and purification process is the careful characterization and identification of a chemical entity within the crude extract having activities that alter a biological activity of the novel CFR-1 isoform described herein. The same *in vivo* and *in vitro* assays described herein for the detection of activities in mixtures of compounds can be used to purify the active component and to test derivatives thereof. Methods of fractionation and purification of such heterogenous extracts are known in the art.

The following examples are provided for the purpose of illustrating the invention and should not be construed as limiting.

Example 1
Materials and Methods

Cell culture and antibody purification

In all assays, the known gastric adenocarcinoma cell line 23132 was used, which is deposited under No. ACC201 at the DSMZ-German Collection of Microorganisms and Cell Cultures GmbH, Mascheronder Weg 1b, 38124 Braunschweig, Germany. Cells were grown to 80% confluency in RPMI-1640 (PAA, Vienna, Austria) supplemented with 10% FCS and penicillin/streptomycin (1% for

both). For the assays described, cells were detached with trypsin/EDTA and washed twice with phosphate buffered saline (PBS) before use. The PAM-1 antibody producing human hybridoma cell line 103/51 was grown in cell culture flasks (175 cm^2) in serum free AIM®V medium (Life Technologies, Karlsruhe, Germany). Cell culture supernatant was collected and the IgM antibody PAM-1 was purified on a HiTrap™ IgM affinity column (Amersham Pharmacia Biotech, Freiburg, Germany) using an FPLC system. The antibody was eluted with 20 mM sodium phosphate, pH 7.5 in 30% isopropanol. Buffer exchange with PBS was performed using NAP™-10 columns (Amersham Pharmacia Biotech). Purity was determined by SDS gel electrophoresis and Western blotting.

Preparation of membrane extracts

Isolation of membrane proteins from tumor cells was performed as described by Hensel *et al.* (Int. J. Cancer 81:229-235 (1999)), using cell line 23132. In short, confluent tumor cells were washed twice with phosphate buffered saline ("PBS"), harvested with a cellscraper and centrifuged, and resuspended in hypotonic buffer (20 mM HEPES, 3 mM KCl, 3 mM $MgCl_2$). After 15 minutes incubation on ice, followed by sonification for 5 minutes, the nuclei were pelleted by centrifugation at 10,000g for 10 minutes. The supernatant was centrifuged for 30 min at 100,000g in a swing-out rotor to pellet membranes. After washing the pellet with hypotonic buffer, it was resuspended in membrane lysis buffer (50 mM HEPES pH 7.4, 0.1 mM EDTA, 10% glycerol, and 1% TRITON X-100). A protease inhibitor (Boehringer, Mannheim, Germany) was added to all solutions.

Western blotting

10% reducing SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis) gels and Western blotting of proteins were performed using standard protocols as described, for example, in Hensel *et al.* (Int. J. Cancer 81:229-235 (1999)). In short, blotted nitrocellulose membranes were blocked with PBS containing 2% low fat milk powder, followed by a one-hour incubation with 10 µg/ml purified primary antibody, e.g., 103/51 or control antibody (intact or fragmented).

The secondary antibody (peroxidase-coupled rabbit anti-human IgM antibody (Dianova, Hamburg, Germany)) was detected with the SUPERSIGNAL chemiluminescence kit from Pierce (KMF, St. Augustin, Germany). After three washes with PBS + 0.05% Tween-20, the blots were incubated with the secondary antibody (peroxidase-coupled rabbit antihuman IgM antibody (Dianova, Hamburg, Germany)). The reaction was detected with the aid of the SUPERSIGNAL chemiluminescence kit from Pierce (KMF, St. Augustin, Germany).

Purification of the antigen 103/51

Purification of the antigens was performed by column chromatography using a Pharmacia (Freiburg, Germany) FPLC unit. For size exclusion chromatography, a Pharmacia SUPERDEX 200 column (XK16/60) was loaded with 5 mg membrane preparation and run with buffer A (100 mM Tris/Cl, pH 7.5, 2 mM EDTA, 40 mM NaCl, 1% Triton X-100). Then, the eluate was fractionated and examined in Western blot analysis for reaction with antibody 103/51. Positive fractions were loaded on a MONOQ (5/5) purification column using buffer A. The bound proteins were eluted with a linear gradient using buffer B (100 mM Tris/Cl, pH 7.5, 1 M NaCl, 2 mM EDTA, 1 M NaCl, 1% Triton X-100), fractionated and examined in Coomassie-stained SDS-PAGE and Western blot analysis. Positive bands were cut out from gel and sequenced or used for immunization of mice.

MALDI peptide mapping

The band of interest was excised from the SDS-PAGE gel and cut into small pieces of about 1 mm x 1 mm. Gel pieces were washed, reduced with DTT (dithiothreitol), S-alkylated with iodoacetamide, and in-gel digested with trypsin (unmodified, sequencing grade, Boehringer) as described, e.g., in Shevchenko *et al.* (Anal.Chem. 68:850-858 1996)). After 3 hours of digestion at 37°C, 0.3 µl of the digest solution was removed and subjected to MALDI peptide mass mapping on a Bruker Reflex MALDI-TOF equipped with delayed extraction (Bruker-Franzen, Bremen, Germany). The thin film technique was adopted for sample preparation (Jensen *et al.*, Rapid.Commun.Mass.Spectrom. 10:1371-1378 (1996)). The tryptic

peptide masses were used to search a non-redundant protein sequence database by the PeptideSearch software program developed in-house.

Cloning of CFR-1 anti-sense vector and transfection

RNA isolation, cDNA synthesis, and PCR were performed using standard methods, as described, e.g., in Hensel *et al.* (Int.J.Cancer 81:229-235 (1999)). In short, for PCR for amplification of a 897 bp fragment ranging from basepairs 802 to 1699, the following primers were used: CFR-For 5' GCTTGGAGAAAGGCCTGGTGAA 3' (SEQ ID NO:9), CFR-Rev 5' TGGCACTTGCGGTACAGGACAG 3' (SEQ ID NO:10). Amplification was performed using the following cycle profile: 95°C, 2 minutes, followed by 35 cycles of 94°C, 30 seconds; 60°C, 30 seconds; 72°C, 60 seconds, and a final extension of 72°C for 4 minutes. Cloning into the pCR-Script Amp SK (+) vector and DNA sequencing were performed as described, e.g., in Hensel *et al.* (Int. J. Cancer 81:229-235 (1999)). The insert was subcloned into the pHook-2 vector (Invitrogen, Leek, Netherlands), and the accuracy of the cloning was verified by sequencing.

Transfection of cell line 23132 with pHOOK2-antiCFR-1 was accomplished with PRIMEFECTOR reagent (PQLab, Erlangen, Germany) according to supplier's manual. In short, plasmid DNA was diluted to 10 μg/ml and the PRIMEFECTOR reagent was added in a 1:10 ratio to a serum-free growth medium. Diluted plasmid DNA (450 μl), diluted PRIMEFECTOR reagent (90 μl), and serumfree medium (460 μl) were mixed and incubated at room temprature ("RT"). 60-milliliter cell culture plates (70% confluent) were washed two times with serumfree medium, and then the PRIMEFECTOR/DNA mixture was added dropwise. Cells were incubated 18 hours at 37°C and 7% CO_2, then serumfree growth medium was replaced with growth medium containing 10% FCS, and cells were incubated another 24 hours before studying CFR-1 expression.

Flow cytometry

The cell line 23132 was detached from culture plates by trypsin /EDTA 48 hours after transfection, washed and subsequently incubated on ice with antibody 103/51 and a human isotype-matched control antibody (Chromopure human IgM) for 15 minutes, followed by incubation with a FITC-labeled rabbit anti-human IgM antibody (Dianova) for 15 minutes on ice. Antibodies were optimally diluted in PBS containing 0.01% sodium azide. Cells were analyzed by flow cytometry (FACScan; Becton Dickinson, USA).

Glycosidase assays

Detached and washed cells were resuspended in RPMI-1640 containing 10% FCS and incubated for 1 hour on ice, then counted, and cytospins were prepared. After air-drying, cytospin preparations were acetone-fixed (10 min), washed, and incubated with 20 μU/ml O-glycosidase or 5 mU/ml N-glycosidase (Boehringer) for 4 hours at 37°C. Slides then were washed and immunohistochemically stained.

For deglycosylation of membranous proteins, membrane extracts were incubated for 16 hours at 37°C with 1 mU/ml N-glycosidase diluted in deglycosylation buffer (50 mM PO_4-Buffer, pH 7.4). As a control, extracts were incubated with deglycosylation buffer alone. Extracts then were separated by SDS-PAGE and Western blots were performed as described above.

Production of murine monoclonal antibodies

BALB/c mice were immunized two times within 17 days with 5 μg purified antigen of antibody 103/51, and killed 4 days after the second immunization. Spleens were disrupted mechanically and fused with 1 x 10^7 NS0 cells as described, e.g., in Vollmers *et al.* (Cell 40:547-557 (1985)). Antibody-producing hybridomas were tested through immunohistochemical staining and reaction in Western blot analysis. Clone 58/47-69 with positive reactivity was used for further experiments.

Immunohistochemical staining of paraffin sections

Paraffin-embedded human gastric mucosa and tumor were sectioned (5 µm), deparaffinized, and blocked with BSA (bovine serum albumin) (15 mg/ ml) diluted in PBS for 30 minutes. The sections were incubated with supernatant of hybridoma 103/51, or 58/47-69, Ki67 (Loxo, Dossenhein, Germany) or mouse anti-cytokeratin 8 antibody diluted 1:15 with BSA/PBS (Dako, Hamburg, Germany) for 2 to 2.5 hours in a humidified incubator. The sections then were washed three times with Tris/NaCl, followed by incubation with peroxidase-labeled rabbit anti-human or rabbit anti-mouse conjugate (Dako) diluted 1:50 in PBS containing rabbit serum (for antibody 103/51) or in PBS containing human AB plasma (for antibody 58/47-69 and anti-cytokeratin). After washing three times with Tris/NaCl and incubation in PBS for 10 min staining was performed with diaminobenzidine (0.05%)-hydrogen peroxide (0.02%) for 10 min at RT. The reaction was stopped under running tap water, and sections were counterstained with hematoxylin.

Immunohistochemical staining of living and acetone-fixed cells

For living cell staining, cells were detached, washed and diluted to $1x10^6$ cells/ml. 1 ml of cell suspension was centrifuged at 1,500g for 5 minutes. Antibody diluted to 40 µg/ml with complete RPMI was added to a final volume of 1 ml and incubated for 90 minutes on ice. Cells then were pelleted at 1,500g for 5 minutes and resuspended with 500 µl RPMI. With 200 µl of the cell suspension, cytospin preparations were prepared and air-dried for 30 minutes. Cells were fixed in acetone for 30 minutes and washed with Tris/NaCl three times. HRP-coupled rabbit anti human IgM (DAKO) was diluted 1 : 50 in PBS/BSA (0.1 %) and incubated for 30 minutes at RT. After three washings, staining was performed as mentioned above.

For staining of acetone-fixed cells, cytospins were prepared, air-dried at RT and fixed in acetone as described above. Cytospins then were blocked for 15 minutes with PBS/BSA (0.1 %) and incubated for 30 minutes with 10 µg/ml primary antibodies followed by three washings. Incubation with secondary antibody and staining was performed as described above.

MTT-proliferation assay

The MTT-assay with the established cell line 23132 was performed as described, e.g., in Vollmers *et al.* (Cancer 74:1525-1532 (1994)). In short, trypsinized cells were diluted to $1x10^6$ cells/ml in complete growth medium, and 50 µl of cell suspension was added to each well of a 96-well plate. 50 µl of the antibody, diluted to the indicated concentrations with complete growth medium, were added to the wells, and plates were incubated for one or two days at 37°C in a humidified incubator. For analysis, 50 µl of MTT (3(4,5 dimethylthiazol)-2,5 diphenyltetrazolium bromide) solution (5 mg/ml) were added to each well, and plates were incubated for 30 minutes. After incubation, plates were centrifuged at 800g for 5 minutes, MTT solution was removed, the stained cell pellet was dissolved in 150 µl dimethylsulphoxide, and absorption was measured at wavelengths of 540 nm and 690 nm.

Cell-Death ELISA

The extent of antibody-induced apoptosis on tumor cell line 23132/87 was analyzed by the Cell Death Detection ELISA^{PLUS} Kit (Roche, Mannheim, Germany). For this assay 1 x 10^4 tumor cells were plated on 96-well plates and incubated in presence of fragmented PAM-1 antibody for 24 hours at 37 °C and 7% CO_2 in a humidified CO_2 incubator. To demonstrate normal growth, the cells were supplemented with complete growth medium (control 1). Unrelated fragmented IgM served as a negative control (control 2). After incubation the cells were centrifuged for 10 minutes at 200 g, the supernatants were removed followed by an incubation with lysis-buffer for 30 minutes at RT. After centrifugation, the supernatants were transferred into a streptavidin-coated microtitre plate (MTP), immunoreagent added (mixture of 10% Anti-Histone-Biotin, 10% Anti-DNA-peroxidase (Anti-DNA POD) and 80% incubation buffer) and incubated for 2 hours at RT on a MTP shaker at 250 rpm. Following incubation, unbound components were removed by washing with incubation buffer. Peroxidase is determined photometrically with an ABTS™ as a substrate (1 ABTS™ (2,2'-Azino-di[3-ethyl-benz-thiazolin-sufonat) tablet in 5 ml

substrate buffer). The antibody induced apoptosis was measured at 405 nm against ABTS solution as a blank (reference wavelength approx. 490 nm).

In vivo experiments

To determine the effects of fragmented PAM-1 on tumor cell growth *in vivo*, the nude mouse-human stomach carcinoma cell system was used (see, e.g., Vollmers et al., Oncology Reports 5:35-40 (1998)). Briefly, 2 x 10^6 stomach carcinoma cells (23132/87) were injected i.p. into 8 week old NMRI nu/nu mice (Harlan Winkelmann GmbH, Borchen, Germany) followed by injections of fragmented PAM-1 antibody (200 μg) at day 4 post carcinoma cell injection. Control mice were injected with unrelated fragmented human IgM in the same concentration. Visible tumor growth was measured macroscopically during the experiment. The experiments were terminated when tumors had reached maximal tolerable size, whereupon the mice were sacrificed, tumor size, respectively tumor weight, was determined, and organs and tissues inspected for the spread of tumors and other alterations.

FragEl-Klenow apoptosis assay

To investigate whether or not the tumors induced in mice were undergoing apoptosis, DNA-fragmentation was measured using immunohistochemistry. The Klenow-assay, which is specific for apoptosis, which is standard in the art and was performed as described in Vollmers et al. (Oncology Reports 5:549-552 (1998)). Briefly, mouse tumors grown in PAM-1 treated mice and in the control group were fixed in 3% formaldehyde and embedded in paraffin. Sections were then processed using the apoptosis-specific FragEL-Klenow DNA Fragmentation Kit (Calbiochem-Novabiochem, Bad Soden, Germany) according to the manufacturer's instructions.

Methods of determining the sequence of novel isoform of CFR-1

RNA was prepared for the cDNA synthesis with the aid of the RNEASY kit from Quiagen. For this RNA preparation, 1x10^6 cells were washed twice using ice cold PBS and pelletized at 1000 x g for 5 minutes and the RNA was prepared in accordance with the manufacturer description. 5 μg RNA (1-5 μl solution) was mixed with 1 μl oligo-dT_{15} (1 μg/μl) and 2 μl random primer (40 μM) and brought up to a

total volume of 8 µl using H_2O. The RNA was denatured for 10 minutes at 65°C and the sample was subsequently cooled on ice. 17 µl Mastermix, consisting of 5.2 µl DEPC-treated H_2O, 5 µl 5x reverse transcriptase buffer, 2.5 µl dNTPs (per 10 mM), 2.5 µl DTT (250 mM), 0.8 µl RNasin (400 U), and 1 µl M-MLV reverse transcriptase (200 U), was then added to the RNA. The synthesis of the cDNA was performed for 70 minutes at 37°C and was subsequently terminated by heating to 95°C for 5 minutes. 1-5 µl of the cDNA was mixed with the PCR Mastermix and brought up to 25 µl total volume using H_2O. The PCR Mastermix consisted of 2.5 µl 10x Taq-polymerase buffer, 0.5 µl 10 mM NTPs, 1.5-2 µl 25 mM $MgCl_2$, 0.5 µl each 20 pM 3' and 5' primer, and 0.2 µl Taq polymerase (1 U). The amplification conditions for the various PCR products are shown in the following overview of the PCR program used for amplifying the various cDNAs.

Product	Annealing in [°C]	$MgCl_2$ [mM]	Extension time [seconds]	Cycles	Product size [bp]
Fragment 1	55	1.75	45	40	691
Fragment 2	60	1.5	45	40	898
CFR Fragment 3	55	2.0	45	40	739
Fragment 4	55	2.0	45	40	941
Fragment 5	55	2.0	45	40	750

Primer sequences

Sequences for the oligonucleotides used for the PCR are shown below

CFR

CFR-For 1 5′ OGC AGC TTC AGC AGC AAC AGC A 3′ (SEQ ID NO:11)

CFR-Rev 1 5′ CAG CTC AGC CAC CCG GAG AAT G 3′ (SEQ ID NO:12)

CFR-For 2 5′ GCT TGG AGA AAG GCC TGG TGA A 3′ (SEQ ID NO:13)

CFR-Rev 2	5′ TGG CAC TTG CGG TAC AGG ACA G	3′ (SEQ ID NO:14)
CFR-For 3	5′ GAA CAC CGT CTC TTA GAG CTG C	3′ (SEQ ID NO:15)
CFR-Rev 3	5′ GCT TCC TGC AGA GTG TCA TTG C	3′ (SEQ ID NO:16)
CFR-For 4	5′ GGA GGA CGT GTT GAA GCT TTG C	3′ (SEQ ID NO:17)
CFR-Rev 4	5′ CCA GGG CAC AAG CAG TAT GAA G	3′ (SEQ ID NO:18)
CFR-For 5	5′ CAA CAG CAG ACA GGT CAG GTG G	3′ (SEQ ID NO:19)
CFR-Rev 5	5′ CCG GAA GTT CTG TTG GTA TGA G	3′ (SEQ ID NO:20)

Sequencing was performed using a sequencer from the firm Applied Biosystems. The following oligonucleotides were used for the sequencing of cloned PCR products:

T_3 5′	ATT TAA CCC TCA CTA AAG GG	3′ (SEQ ID NO:21)
T_7 5′	GTA ATA CGA CTC ACT ATA GGG C	3′ (SEQ ID NO:22)

3 µl plasmid DNA was mixed with 1 µl primer (3.2 pM), 11 µl H_2O, and 5 µl reaction mixture of the ABIPRISM Sequencing Kit and incubated in the thermocycler for 25 cycles using the following parameters:

Denaturing	Annealing	Extension
95°C, 30 seconds	52°C, 15 seconds	60°C, 4 minutes

To remove oligonucleotides and dNTPs, the reaction mixture was purified via a Sephadex G-50 filled purification column. For this purpose, a 100 µl pipette tip was loaded up to the upper edge with column material and centrifuged for 3 minutes at 2000 x g. Subsequently the sample was applied and the small column was centrifuged again. The DNA was then precipitated by 2 µl Na acetate (pH 5.2) and 50 µl 100% ethanol and pelletized by centrifuging at 13,000 x g for 15 minutes. After drying, the DNA was received in 3 µl formamide/25 mM EDTA (5:1) and analyzed in the sequencer.

Sequence Analysis

At least five clones were sequenced from each cloning reaction. To identify potential errors which arose during the amplification using the Taq-polymerase and/or the sequencing, the sequences of the cloned PCR fragments were compared with one another with the aid of the DNAsis for Windows software and a consensus sequence of all clones was established from both read directions. By rewriting the DNA sequences into amino acid sequences, the number of silent mutations and amino acid substitution mutations were determined. The sequences for MG160 and CFR-1 were drawn from the NCBI databank and compared to sequence of the PCR products using the DNAsis for Windows program. An alignment of these sequences is shown in Figure 16.

RNA-Isolation

RNA from normal and cancerous gastric tissue of the stomach was isolated using the phenol-guanidine-isothiocyanate method with TRIZOL® Reagent (Invitrogen). In brief, frozen normal and tumor tissues were cut in serial 5µm sections on a freezing microtome. 1 ml TRIZOL® Reagent was added to the tissue samples and the solutions were homogenized subsequently. Following homogenization the insoluble material was removed from the homogenate by centrifugation at 12,000 x g for 10 minutes at 4°C. 200µl chloroform was added to the RNA containing supernatant, and, after mixing the solution was incubated for 3 minutes at RT. After centrifugation for 15 minutes at 12,000 x g and 4°C, the aqueous phase was

precipitated in 500 µl isopropanol by mixing for 30 secods, incubation for 10 minutes at RT and centrifugation for 10 minutes at 12,000 x g and 4°C. The resulting RNA pellet was washed with 1ml of 75% ethanol and centrifuged for 5 minutes at 7,500 x g at 4°C. The RNA pellet was air-dried and re-suspended in 80µl DEPC-treated water. The integrity and quality of purified total RNA were controlled by 1% agarose gel electrophoresis and the concentrations were evaluated by spectrophotometry.

Semi-quantitative Reverse Transcription-PCR

mRNA levels were examined using semi-quantitative Reverse Transcription-PCR (RT-PCR) method. Synthesis of first-strand cDNA from normal and cancerous gastric tissue was performed with 5µg of total RNA using Moloney murine leukemia virus reverse transcriptase (M-MLV RT, Invitrogen GmbH, Karlsruhe, Germany) and oligo-dT primer according to the supplier's manual. The PCR method was used to detect CFR-1 mRNA. PCR reactions were carried out in a 25µl volume with 2 nM $MgCl_2$, 0,4 pM primer, 200µm each dNTP and 1 unit of *Taq* polymerase (MBI). The expression of CFR-1 mRNA was normalized to GAPDH mRNA levels. The primers specific for CFR-1 and GAPDH were designed on their reported sequences and commercially synthesized by MWG-BIOTECH AG (Ebersberg, Germany). The sequences of these oligonucleotides are 5' CAAGAGCAGACAG-GTCAGGTGG 3' (SEQ ID NO:22) and 5' CCGGAAGTTCTGTTG-GTATGAG 3' (SEQ ID NO:23) for CFR-1 and 5' GTGGAAGGACTCATGACCACAGTC 3' (SEQ ID NO:24) and 5' CATGTGGGCCATGAGGTCCACCAC 3' (SEQ ID NO:25) for GAPDH. Sizes of expected amplification products are 750 bp for CFR-1 and 482 bp for GAPDH. CFR-1 was amplified at 94°C for 4minutes and for 40 cycles at 94°C (30 s), 55°C (30 s) and 72°C (30 s) with a final extension step at 72°C (4 min). As a negative control each PCR run included a sample containing PCR buffer but no cDNA. The PCR products were identified by agarose-gel-electrophoresis (2%) in Tris-acetate-EDTA buffer and ethidium bromide staining.

Pepsin cleavage

For pepsin digestion of the PAM-1 antibody a buffer exchange with 100mM sodium citrate (pH 3.5) using NAP™-10 columns (Amersham Pharmacia Biotech) was used. Additionally pepsin digestion was done with an unrelated human IgM antibody (Chrompure IgM, Dianova, Hamburg, Germany) to obtain a suitable negative control. For each milligram of antibody, 5 µg pepsin (Sigma Aldrich, Taufkirchen, Germany) was added, followed by incubation for 10-15 minutes in a 37 °C water bath. The reaction was stopped by adding 1/10 volume of 3.0 M Tris (pH 8.8) followed by centrifuging at 10,000 g for 30 minutes. Prior to use in experiments the fragmented PAM-1 antibody and the fragmented human control IgM were dialyzed against PBS. The success of pepsin cleavage was examined by SDS gel electrophoresis and Western blotting.

The following experiments were carried out using the above materials and methods.

Example 2
Identification and characterization of the antigen recognized by the PAM-1 antibody

Purification and identification of antigen 103/51

Western Blot analysis was used to show that the antibody 103/51 binds to an approximately 130 kD membrane protein on stomach cancer cells. We pre-purified this protein by sequential size exclusion and anion exchange chromatography (Fig. 1A). The protein was excised from a Coomassie-stained preparative SDS-PAGE, one part was used for production of mouse monoclonal antibodies (see below), and one part was used to identify the protein using the methods standard in the art, as outlined by Shevchenko et al. (Proc. Natl. Acad. Sci. U.S.A. 93:14440-14445 (1996)). After 3 hours of in-gel digestion with trypsin, about 1% of the total digested volume was removed and subjected to high mass accuracy MALDI peptide mass mapping (saving the rest of the digest for nanoelectrospray analysis, in case MALDI MS did not lead to definitive identification). Despite the femtomole amount of the protein digest consumed for MALDI analysis, a database search matched 35 peptides to the CFR-1 sequence with a mass accuracy within 50 ppm. These peptides cover 29% of the CFR-

1 sequence, thus definitively identifying the protein as a CFR-1 homologue. CFR-1 has a calculated molecular weight of approximately 134 kD (Burrus *et al.*, 1992, Mol. Cell Biol. 12:5600-5609) (Fig. 1B). The nucleic acid (SEQ ID NO:5) and amino acid (SEQ ID NO:6) sequence of this novel CFR-1 homologue are shown in Figures 10-1 to 10-5.

Effect of transient transfection of cell line 23132 with CFR-1 antisense vector on binding of antibody 103/51 and live cell staining

We investigated the effect of an antisense transfection of the stomach carcinoma cell line 23132 using immunohistochemistry and flow cytometry. For this, an 897 bp PCR-fragment of CFR-1, flanking the region between basepairs 802 and 1699, was cloned into the pHOOK-2 vector in an antisense direction in reference to the CMV promoter. The washed cells were transfected with the pHOOK-CFR anti-sense vector, pHOOK-lacZ, and pHOOK vector in an intermediate step. Transfection was controlled by a β-Galactosidase assay (data not shown). 48 hours after transfection, cytospin preparations were prepared and stained with antibodies 103/51 and anti-cytokeratin 18 as a control (data not shown).

The immunohistochemistry showed a clear reduction of staining in cells transfected with the pHOOK-CFR antisense vector when compared to mock-transfected cells (Figs. 2A and 2B). This confirmed the binding of antibody 103/51 to CFR-1. The slight cytoplasmic staining visible in both stainings might be due to non-specific binding often observed in staining with human IgM antibodies on acetone-fixed cells. Membrane expression and the effect of transfection were also tested by flow cytometry (Figs. 2G-2I). The data indicate a reduction in binding of antibody 103/51 after transfection of cells with the CFR-1 antisense vector. However, untreated cells or cells transfected with the control vector pHOOK-2 shows a clear binding to cell line 23132, indicating expression of a CFR-1 isoform on the cell membrane.

To investigate the specific membrane distribution of the CFR-1 isoform, we performed live cell staining with cell line 23132 and some non-stomach cancer cell lines. On the cell line 23132 we found a clear staining (Figs. 2C and 2D), while the

human lung adenocarcinoma cell lines Colo-699 (Figs. 2E and 2F) and human epidermoid lung carcinoma cell line EPLC-272H (data not shown) were clearly negative. These data show that the described CFR-1 isoform is not expressed in all cancerous cell lines, and the exclusive membrane staining of 23132 cells indicates that the CFR-1 isoform has a distribution that is different from the one described so far for CFR-1.

Glycosidase assay

CFR-1 is a sialoglycoprotein with 5 possible N-glycosylation sites and it has been shown by treatment with glycosidase F that the molecule is glycosylated at these sites (Steegmaier *et al.*, Nature 373:615-620 (1995)). Since tumor-reactive antibodies often react with carbohydrate residues, we investigated whether this is the case for the antibody 103/51. Cytospin preparations of cell line 23132 were incubated for 4 hours with O- and N-glycosidases, and then subjected to immunohistochemical staining with antibody 103/51. Treatment of cells with N-glycosidase led to a dramatic decrease in 103/51 staining (Fig. 3B), while incubation with dephosphorylation buffer (Fig. 3A) or digestion with O-glycosidase (data not shown) had no effect on binding of the antibody 103/51. This shows that the specificity of binding of the antibody 103/51 must be located in N-linked sugar residues and not in the primary protein sequence.

To further control for this effect, membrane extracts of cell line 23132 were deglycosylated for 16 hours and Western blots were prepared and stained with antibody 103/51. We found a reduction in the reaction on lysates incubated with N-glycosidase when compared to the control lysates (Fig. 3C).

Production of murine antibodies and immunohistochemical staining of paraffin section of stomach adenocarcinoma

Commercial antibodies which specifically recognize CFR-1 are not available. Thus, we immunized mice with purified protein eluted from Coomassie-stained SDS-gel for production of monoclonal antibodies to strengthen the specificity, and to further characterize CFR-1 expression. Spleen cells were immortalized by fusion with the heteromyeloma NS0. 150 clones were tested for immunohistochemical staining.

Positive clones were re-cloned, and the clone 58/47-49 (IgM) was used for further characterization. To investigate the binding properties of the human antibody 103/51 and the murine antibody 58/47-69, we stained paraffin sections of 15 different stomach adenocarcinoma and one adenoma. Identical staining of glandular cells of the normal epithelial tissue and intensive staining of carcinoma cells was found (Figs. 4A-4D). In short, early carcinoma (n = 2) were stained by both antibodies. On interstitial-type carcinoma both antibodies stained 4 out of 5 cases, on diffuse-type carcinoma all cases (n = 4) were stained, and the intermediary-type were positive in 50 % (n = 4) with both antibodies. These results show a high expression of the CFR-1 isoform described herein in most cases of stomach carcinoma. The investigated adenoma showed a distinct staining pattern, with positive cells only in the transition from normal to transformed cells.

The nucleic acid sequence (SEQ ID NO:1) and the amino acid sequence (SEQ ID NO:2) of the variable region of the heavy chain of murine antibody 58-49/69 are shown in Figures 8A and 8B. As indicated in Figure 8B, CDR1 of the 58-49/69 variable region heavy chain spans nucleotides 31-45 which encode amino acids 11-15, CDR2 spans nucleotides 88-138 which encode amino acids 30-46, and CDR3 spans nucleotides 235-264 which encode amino acids 79-88. In addition, the D-gene spans nucleotides 235-243 and the J-gene spans nucleotides 243-288.

The nucleic acid sequence (SEQ ID NO:3) and the amino acid sequence (SEQ ID NO:4) of the variable region of the light chain of murine antibody 58-49/69 are shown in Figures 9A and 9B. As indicated in Figure 9B, CDR1 of the 58-49/69 variable region light chain spans nucleotides 49-96 which encode amino acids 17-32, CDR2 spans nucleotides 142-162 which encode amino acids 48-54, and CDR3 spans nucleotides 259-285 which encode amino acids 87-95.

The nucleic acid sequence (SEQ ID NO:28) and the amino acid sequence (SEQ ID NO:26) of the variable region of the heavy chain of human antibody 103/51 are shown in Figure 17. CDR1 of the 103/51 variable region heavy chain spans nucleotides 31-54 which encode amino acids 11-18, CDR2 spans nucleotides 106-129 which encode amino acids 36-43, and CDR3 spans nucleotides 244-312 which encode amino acids 82-104.

The nucleic acid sequence (SEQ ID NO:29) and the amino acid sequence (SEQ ID NO:27) of the variable region of the light chain of human antibody 103/51 are shown in Figure 18. CDR1 of the 103/51 variable region light chain spans nucleotides 82-96 which encode amino acids 28-32, CDR2 spans nucleotides 151-159 which encode amino acids 51-53, and CDR3 spans nucleotides 268-300 which encode amino acids 90-100.

Immunohistochemical staining with antibody 103/51 on gastric mucosa

To investigate the reaction pattern of antibody 103/51 on gastric mucosa in more detail, we performed immunohistochemical stainings on gastric tissue without inflammation, *H. pylori* associated chronic active gastritis, high-grade dysplasia, and gastric adenocarcinoma (Figs. 5A-5D). On non-inflammed gastric tissue no reaction was seen. However, in the mucosa of a patient with *H. pylori* gastritis we found staining predominantly in the basal zone of foveolar cells. The staining pattern of antibody 103/51 shows a strong correlation with the activation pattern shown by Ki67 staining (Ramires *et al.*, 1997, J. Pathol. 182:62-67). A more intensive staining of antibody 103/51 was seen in the proliferation zone of gastric dysplasia also correlating with Ki67 staining. The strongest staining was found in the proliferating zone of gastric adenocarcinoma.

Immunohistochemical staining of antibodies 103/51 and 58/47-69 on different tissues

We investigated the expression of the CFR-1 isoform described herein in other cancerous and normal tissues by immunohistochemical staining of paraffin sections with antibodies 103/51 and 58/47-69. Out of 15 cancerous tissues (other than stomach carcinoma), antibody 103/51 showed staining in 13 cases (Figs. 6A-6F, Table 1A). Negative staining was observed on anaplastic cells of the lung, confirming the results from the immunohistochemical staining and MTT-assay with the cell lines Colo-699 and EPLC-272H. These data indicate an over-expression of the CFR-1 isoform described herein and distribution to the cell membrane in malignant transformed cells.

Table 1A: Reaction pattern of antibody 103/51 with different tumor tissues.

Tissue	Carcinoma-Type	Antibody-Staining
Esophagus	Squamous	+
Stomach	Adeno (diffuse)	++
Stomach	Adeno (interstitial)	+
Colon	Adeno	+
Rectum	Adeno	+
Liver	Adeno (HCC)	++
Gallbladder	Adeno	+
Pancreas	Adeno (ductal)	+
Papilla of Vater	Adeno	+
Lung	Large cell anaplastic	-
Lung	Small cell	-
Lung	Adeno	++
Bronchus	Squamous epithelium	+
Mamma	Invasive (ductal)	+
Mamma	Invasive (lobular)	+

Table 1B: Reaction pattern of antibody 103/51 with different normal tissues and pre-cancerous lesions.

Tissue	Cell Type	Antibody-Staining
Salivary gland	Glandular	-
Stomach (not inflamed)	Glandular	-
Stomach (*H. pylori* infected)	Glandular	+[1]
Stomach (high grade dysplasia)	Glandular	++[2]
Duodenum	Glandular	-
Colon	Epithelial	-
Rectum	Glandular	-
Pancreas	Glandular	-
Liver	Glandular	-
Gallbladder	Glandular	-
Oral mucosa	Squamous epithelium	-
Anal mucosa	Squamous epithelium	-
Skin	Keratinocyte, glandular	-
Mamma	Glandular	-
Larynx	Epithelial	-
Bronchus	Epithelial	-
Lung	Glandular, alveolar	-
Thyroid gland	Glandular	-
Adenohypophysis	Glandular	-
Adrenal gland	Glandular	++[2]
Testis	Glandular	-
Ovary	Glandular	-
Prostate	Glandular	-
Urothelium	Epithelial	-
Kidney	Epithelial	++[3]
Thymus	Lymphatic	-
Spleen	Lymphatic	-
Lymph node	Lymphatic	-
Cerebral cortex	Neural	-
Peripheric neural ganglia	Neural	-

For Tables 1A and 1B, antibody staining was scored as follows: "-" = no staining; "+" = moderate staining; "++" = intense staining. HCC = hepatocellular carcinoma, [1] Proliferation zone, Glandular foveola, [2] Glomerular, fascicular zone (membranous staining), [3] Collection tubes of the endoplasmic reticulum.

On 28 normal and pre-cancerous tissues tested, we found a restricted expression only on three interstitial organs (Table 1B). Membrane staining was observed on the glandular foveola of the stomach and the glomerular and fascicular zones of the adrenal gland, while staining of the Golgi apparatus was found in the collection tubes of the kidney (Fig. 5). The Golgi specific staining further confirms the characterization of the antigen as homologous to CFR-1 that has been described earlier by Burrus et al. (Mol. Cell Biol. 12:5600-5609 (1992)).

Stimulation with human and murine monoclonal antibodies

Antibody 103/51 leads to the stimulation of cell line 23132 *in vitro*. We measured this stimulation of antibody 103/51 using the mitochondrial hydroxylase assay (MTT), which is a standard assay for proliferation (Carmichael *et al.*, Cancer Res. 47:936-942 (1987)). To further investigate the stimulating properties of antibody 103/51, we incubated the cell line 23132 with various concentrations of purified antibody. We found a concentration-dependent stimulation with the highest activity at 4 µg/ml (Fig. 7A). Higher concentrations showed a slight decrease in stimulation.

To test if the murine antibody 58/47-69 has the same effects on cell growth, we performed the MTT-stimulation assay with purified antibodies in comparable amounts. As it can be seen in Fig. 7B, both antibodies lead to the stimulation of cell line 23132 *in vitro*. This further confirms identical specificity of both antibodies.

To confirm that the stimulation of antibody 103/51 and the murine antibody 58/47-69 is mediated by binding to the CFR-1 isoform described herein, we transfected cells with control vector pHOOK-2 and CFR-1 antisense vector and tested transfected cells in the MTT-assay. As a positive control for transfection, cells were also transfected with pHOOK-2-lacZ vector followed by ß-galactosidase staining (data not shown). Given that comparable stimulation was observed in non-transfected cells

and cells transfected with control vector pHOOK-2, a reduction of the stimulating effect of both antibodies by the transfection procedure can be excluded. In contrast, cells transfected with CFR-1 antisense vector clearly show a reduced stimulation (Fig. 7C).

Finally, to demostrate that the stimulation by antibody 103/51 is not mediated by receptors other than the CFR-1 isoform described herein, we performed a MTT-stimulation assay with cell line the 23132 and compared it with lung carcinoma cell lines Colo-699 and EPLC-272H that do not express this isoform. While the cell line 23132 is stimulated as described above, the two lung carcinoma cell lines do not show any stimulation by antibody 103/51 (Fig. 7D), confirming the results observed in the immunohistochemistry.

Example 3
The antigen recognized by the PAM-1 antibody is specifically expressed on cancerous and pre-cancerous cells

To further study and illustrate the highly specific expression of CFR-1 isoform recognized by the PAM-1 antibody on malignant tissue, 5 to 13 different cases of the most frequently occurring carcinomas were tested immunohistochemically. The reactivity of PAM-1 was compared with the expression of the Ki67 protein, which is localized in the nucleus of all proliferating cells. The function of this protein remains unknown (Endl and Gerdes, Exp. Cell Res. 257:231-237 (2000)), but it is the most widely used standard marker for proliferation studies (Scholzen and Gerdes, J. Cell. Physiol. 182:311-322 (2000); and Brown and Gatter, Histopathology 40:2-11 (2002)). The staining results are summarized in Table 2.

Table 2: Expression of the isoform of CFR-1 recognized by PAM-1 on tumor tissues.

Tissue	Carcinoma type	Sex		Age	Ki67 staining	PAM-1 staining
		m	f		+/-	+/-
Esophagus	Squamous cell	4	1	50-70	4/1	5/0
	Adeno (Barrett)	12	1	48-85	11/2	11/2
Stomach	Adeno (diffuse)	3	2	50-80	2/3	5/0
	Adeno (intestinal)	2	3	68-89	3/2	4/1
	Adeno (Cardia)	7	1	50-74	2/6	8/0
Colon	Adeno	8	5	38-88	10/3	13/0
Liver	Adeno (HCC)	8	1	43-76	0/9	9/0
Pancreas	Adeno (ductal)	5	3	41-75	2/6	8/0
Lung	Adeno	8	3	42-78	2/9	8/3
	Squamous cell	8	1	42-82	5/4	8/1
Mamma	Invasive (ductal)	0	5	37-88	4/1	5/0
	Invasive (lobular)	0	5	40-89	1/4	5/0
Ovary	Adeno	0	8	37-80	8/0	7/1
Uterus	Adeno	0	9	50-80	6/3	8/1
Cervix	Squamous cell	0	10	46-70	3/7	9/1
	Adeno	0	9	33-65	8/1	9/0
Prostate	Adeno	9	0	49-70	1/8	9/0

Taken together, while PAM-1 shows a broad, intensive, and homogeneous staining on all carcinomas, Ki67 is not found in all carcinomas, it shows only a weak expression in most cases, and in contrast to PAM-1, it is non-homogeneously distributed (Table 2). Adenocarcinomas of the liver (HCC) are all negative and only several cases of adenocarcinomas of prostate, lung and invasive lobular carcinomas of the breast are positive for Ki67. These data also strongly confirm that the CFR-1 isoform recognized by PAM-1 is specifically expressed on most tested carcinomas.

Gene expression of CFR-1 in normal and tumor tissue

PAM-1 antibody reacts with a N-linked carbohydrate residue on the isoform of CFR-1 described herein which is specifically found on malignant cells. The lack of PAM-1 binding to non-malignant cells can therefore be either the result of a non-expression of the receptor on normal cells or of a post-transcriptional modification of CFR-1, which is different to that found on malignant cells. We investigated this on molecular level. CFR-1 mRNA of non-malignant and cancerous gastric tissue was examined using semi-quantitative RT-PCR. The RT-PCR method was standardized by using the constitutively expressed "housekeeping gene" GAPDH as an internal control. An increased expression level of CFR-1 could be detected in gastric tumor

tissue compared with normal gastric tissue. This shows that CFR-1 detected by antibody PAM-1 on malignant cells is a specific over-expressed and most likely post-transcriptionally modified isoform of CFR-1.

Expression of the CFR-1 isoform recognized by the PAM-1antibody on pre-malignant tissue

The CFR-1 isoform recognized by the PAM-1 antibody is expressed in stomach cancer precursor lesions like *H. pylori*-induced gastritis and gastric dysplasia and the level of expression likely increases with the escalation of malignancy. To manifest and improve this observation and to obtain a comparison with the proliferation marker Ki67, additional immunohistochemical experiments were performed with a variety of other precursor lesions. The illustrations of precursor lesions from colon, esophagus, cervix, and bronchial carcinoma and corresponding proliferation zones described below demonstrate the specific expression of the PAM-1 antigen. The data are summarized in Table 3.

Table 3: Expression of the isoform of CFR-1 recognized by the PAM-1 antibody on pre-cancerous tissues (nd = not determined).

Tissue	Precursor lesions	Sex		Age	Ki67 staining +/-	PAM-1 staining +/-
		m	f			
Esophagus	Barrett metaplasia	9	0	42-69	8/1	9/0
	Barrett dysplasia	4	2	62-86	3/3	6/0
Stomach	*H. pylori* gastritis	5	5	24-86	7/3	9/1
	Atrophic gastritis	1	2	53-79	0/3	3/0
	Intestinal metaplasia	5	2	49-86	7/0	7/0
	Tubular adenoma	5	4	42-87	6/3	8/1
	Tubulovillous adenoma	2	2	54-84	3/1	3/1
	High grade dysplasia	3	0	65-74	3/0	3/0
Colon	Dysplasia (ulcerative colitis)	4	1	42-57	4/1	5/0
	Tubular adenoma	5	2	54-85	5/2	6/1
	Villous adenoma	8	2	45-85	9/1	8/2
Cervix	CIN I	0	8	22-52	7/1	8/0
	CIN II	0	5	30-62	4/1	5/0
	CIN III	0	5	29-41	5/0	5/0
Bronchus	Squamous metaplasia	5	0	61-72	3/2	5/0
	Epithelial dysplasia	3	0	64-75	3/0	3/0
Breast	D-CIS	1	7	48-78	nd	8/0
	L-CIS	0	3	49-50	nd	3/0
Prostate	PIN	15	0	55-76	nd	14/1

Adenoma-carcinoma sequence

Malignant changes and the resulting carcinomas of the colon belong to the frequently occurring neoplasia and are often associated with high mortality. The formation of colon carcinomas is a multi-step process which could be retraced to the so-called adenoma-carcinoma sequence. All adenomatous lesions arise as a result of epithelial proliferative changes and there is strong evidence that adenomas are a precursor lesion for invasive colorectal adenocarcinoma (Cummings, Semin. Gastrointest. Dis. 11:229-237 (2000); Scheiden et al., Int. J. Colorectal Dis. 15:29-34 (2000); and Wehrmann and Fruhmorgen, MMW Fortschr. Med. 142:26-29 (2000)). As such, colorectal carcinogenesis provides the ideal opportunity to investigate the reactive pattern of the PAM-1 antibody in precancerous lesions in more detail. Additional immunohistochemical stainings were therefore performed on different types of mucosa and epithelia. As noted above, PAM-1 antibody reacts with *H. pylori*-associated chronic active gastritis, high-grade dysplasia and gastric adenocarcinoma. We also determined that the PAM-1 antibody stains atrophic gastritis and intestinal metaplasia, which are pre-cancerous stages in the gastric carcinogenesis. Non-inflamed colon mucosa showed no reaction. Increased expression of the isoform of CFR-1 recognized by the PAM-1 antibody was found in adenomas of the colon, which have a higher risk of degenerating into adenocarcinomas. Expression of the isoform of CFR-1 recognized by the PAM-1 antibody was seen both in tubular and in villous adenomas, particularly in the proliferation zone.

Ulcerative colitis- related dysplasia, which consists of atypical changes in epithelial cells, is also recognized to be involved in the development of colorectal adenocarcinoma (Wong et al., Histopathology 37:108-114 (2000)). On this high grade dysplasia, clear staining by the PAM-1 antibody, especially of these atypical epithelial cells, was observed.

The most intense staining was found in colorectal adenocarcinoma, following the obtained results in case of gastric mucosa. Here, the expression of the isoform of CFR-1 recognized by the PAM-1 antibody correlates with the pattern of Ki67.

Barrett carcinogenesis

Barrett esophagus is a complication of long-standing gastroesophageal reflux. The distal squamous mucosa is replaced by metaplastic columnar epithelium, as a response to prolonged injury. The carcinogenesis of esophagial adenocarcinoma takes place from Barrett metaplasia to Barrett dysplasia (Spechler, Semin. Gastrointest. Dis. 7:51-60 (1996); Haggitt, Hum. Pathol. 25:982-933 (1994); Devesa et al., Cancer 83:2049-2053 (1998); and Spechler, Am. J. Med. 111 Suppl 8A:130-136 (2001)).

Due to the increasing incidence of Barrett carcinoma, the expression of CFR-1 on Barrett epithelium was investigated using immunohistochemical staining with the PAM-1 antibody. Staining with PAM-1 revealed an increased expression of the isoform of CFR-1 recognized by the PAM-1 antibody in the metaplastic columnar epithelium of Barrett metaplasia. In addition, an intense staining pattern was observed in Barrett dysplasia, especially those cells with architectural and cytological abnormalities. The latter are regarded as precursors of the invasive adenocarcinomas of the esophagus (Barrett carcinoma) (Spechler, Am. J. Med. 11 Suppl 8A:130-136 (2001)), and correlated with expression of Ki67. The strongest staining was found in Barrett carcinoma. Although the PAM-1 antibody showed an intense staining pattern for carcinoma of the cardia (heart), Ki67 was not expressed in a comparable manner.

Cervical neoplasia

Increased expression of the isoform of CFR-1 recognized by the PAM-1 antibody on cervical epithelium with architectural abnormalities was also observed. The precursors of the cervical squamous cell carcinoma are classified as cervical intraepithelial neoplasia grade I, II and III. Mild dysplasiae are termed CIN I up to carcinoma *in situ* lesions CIN III (Arends et al., J. Clin. Pathol. 51:96-103 (1998)). The precursor lesions (CIN I-III) and invasive malignancy of the cervix, the squamous cell carcinoma were included in this study.

Normal epithelium showed no reaction with the PAM-1 antibody, whereas increased staining with the PAM-1 antibody was noted for the different types of cervical neoplasia. The staining pattern followed the appearance of atypical cells in the different cell layers and the widening of the basal proliferation zone.

The reaction of Ki67 generally correlated with the staining pattern of the PAM-1 antibody, but the staining was less intense.

Bronchial carcinogenesis

Carcinomas of the lung are one of the most frequently occurring carcinomas world-wide. The most common type is the squamous cell carcinoma, which correlates closely with a history of smoking. In the airways of smokers, squamous metaplasia and dysplasia are usually present. In squamous metaplasia the normal bronchial ciliated epithelium is replaced by squamous epithelium. With occurrence of cytological disturbance and severe atypia, the lesion becomes known as squamous dysplasia (Colby et al., Adv. Anat. Pathol. 5:205-212 (1998); and Franklin, J. Thorac. Imaging 15:3-12 (2000)).

Normal ciliated epithelium shows no expression of the isoform of CFR-1 recognized by the PAM-1 antibody, while the PAM-1 antibody reacted with metaplasia and dysplasia of bronchus epithelium. Squamous cell metaplasia of the bronchus represents the initial stages of carcinogenesis and shows a lower intensity of staining compared to dysplasia. For dysplasia, the preliminary stage of cancer, a more intensive staining was observed. The most intense staining was again observed in the squamous cell carcinoma. In each of the three cases in this study, the staining of PAM-1 correlated with the reaction pattern of Ki67.

Proliferation zones

To investigate whether expression of the isoform of CFR-1 recognized by the PAM-1 antibody is specific for malignant proliferation and not involved in normal proliferation processes (e.g., regeneration of tissue), we stained different proliferative regions of healthy and pre-malignant tissue with PAM-1 and Ki67 antibodies. We observed that the proliferation zone of normal colon mucosa is positive for Ki67, but negative for expression of the isoform of CFR-1 recognized by the PAM-1 antibody. The same result was seen with normal cervical tissue. Here again Ki67 shows a positive staining of the proliferation zone while the isoform of CFR-1 recognized by the PAM-1 antibody is not expressed. In contrast, non-dysplastic intestinal-type

Barrett metaplasia, which defines Barrett's esophagus the pre-malignant lesion for adenocarcinoma of the esophagus, shows a positive expression of Ki67 and the isoform of CFR-1 recognized by the PAM-1 antibody. This clearly shows that the isoform of CFR-1 recognized by the PAM-1 antibody is not expressed in healthy proliferating cells.

Summary of the expression of the isoform of CFR-1 recognized by the PAM-1 antibody on pre-malignant lesions

The immunhistochemical data on precancerous lesions are summarized in Table 3 (above). 3 to 15 different cases of each available precursor lesion type were tested. In general, antibody PAM-1 shows a clear positive and homogeneous staining on nearly all different precursors and in addition an increasing level of expression with the grade of malignancy. In contrast, the proliferation marker Ki67 shows a similar non-homogeneous expression on carcinomas (see, for example, atrophic gastritis, tubular adenoma of stomach, and squamous metaplasia of bronchus) and is expressed on both healthy and malignant tissue. In addition, proliferation zones of healthy tissue are clearly positive for Ki67, but negative for the isoform of CFR-1 recognized by the PAM-1 antibody, supporting the association of this isoform of CFR-1 with malignancy.

Example 4

Fragmented PAM-1 IgM antibody and recombinant PAM-1 IgG induce apoptosis

Apoptosis is the programmed cell death, suicide of cells, through fragmentation of the DNA, cell shrinkage, and dilatation of the endoplasmic reticulum, followed by cell fragmentation and the formation of membrane-bound vesicles, or apoptotic bodies. Apoptosis, the physiological form of cell death, guarantees rapid and clean removal of unnecessary cells, without triggering inflammation processes or tissue trauma, as in the case of necrosis. Under pathological conditions, it is also used for removing malignant cells, such as cancer precursor cells. It may be triggered through greatly varying stimuli, such as through cytotoxic T-lymphocytes or cytokines, such as tumor necrosis factor, glucocorticoids,

and antibodies. It is the most frequent cause of death of eukaryotic cells and occurs in embryogenesis, metamorphosis, and tissue atrophy. Apoptotic receptors on the cell surface, such as those of the NGF/TNF family, are predominantly expressed on lymphocytes, but are also found on various other cell types, wherefore they are not suitable for cancer therapy. In particular, ligands and antibodies for these receptors have led to liver damage in *in vivo* tests. Therefore, tumor-specific receptors having apoptotic function are especially important.

PAM-1 cleavage

Pepsin digestion was used to cleave the intact pentameric PAM-1 antibody into IgM antibody fragments. Following cleavage the resulting fragments were analyzed by SDS-PAGE and Western blotting under non-reducing conditions. After blotting, the intact antibody showed the characteristic bands corresponding to intact antibody, monomeric forms and light chains. By SDS-PAGE, the intact pentameric IgM of about 900 kDa was unable to migrate into the stacking gel. Following 10-15 minutes of treatment with pepsin the pentameric form was completely digested into monomeric, $F(ab)_2$, Fab, and light chain fragments which could be clearly identified by molecular weight (Figs. 11A and 11B). No pentameric form of PAM-1 was left after digestion. The same experiment was done with an unrelated human IgM antibody leading to similar results (data not shown). The unrelated fragmented human IgM was used as a negative control in all following experiments.

The fragmented PAM-1 antibody was tested for tumor-binding on paraffin sections of human stomach carcinomas and precursors and compared to the intact PAM-1. Both antibody forms possess similar binding patterns on tumor and precursor cells as illustrated in Figs. 12A and 12B.

PAM-1 in vitro activity

To define the *in vitro* activity of the fragmented PAM-1 we used the colorimetric mitochondrial hydroxylase assay (MTT). We incubated cell line 23132/87 with various concentrations of the fragmented PAM-1 and found that the fragmented PAM-1 antibody inhibited cell proliferation in a concentration dependent

manner (Fig. 13A). In contrast the cell growth of cells treated with different concentrations of unrelated fragmented IgM is not affected.

To further investigate the inhibitory effect of fragmented PAM-1 antibody on tumor cell growth, the apoptosis-specific Cell Death Detection ELISA^{PLUS} Kit was used. The experiment illustrated in Fig. 13B clearly showed that fragmented PAM-1 antibody inhibits cell growth by inducing apoptosis in stomach carcinoma cells *in vitro*.

Fragmented PAM-1 in vivo activity

To determine the effects of fragmented PAM-1 on tumor cell growth *in vivo*, a nude mouse-human stomach carcinoma cell system was used. A concentration of 2 x 10^6 cells derived from the human stomach carcinoma cell line 23132 were injected intraperitoneal (i.p.) into NMRI nu/nu mice. Four days after the inoculation of tumor cells, 200µg of fragmented PAM-1 antibody, diluted in PBS, was injected i.p. Control mice received the same quantity of unrelated fragmented human IgM. Throughout the duration of the study, tumor growth was controlled macroscopically. After 23 days the mice were sacrificed. The control mice developed measurable tumors i.p. from day 5 onwards. A steep increase in tumor size was observed until day 23. In comparison mice treated with fragmented PAM-1 antibody did not develop detectable tumors before day 10. Moreover, the tumors which developed during the course of the experiment showed a significant reduction in growth and size. (Fig. 14A).

The study was also extended to include an inspection of organs and tissues of the mice for hidden spread of tumors and other alterations. In two additional experiments mice were inoculated i.p. with gastric cancer received fragmented PAM-1 or control antibody. After 23 days mice were inspected for tumor-growth. The control group showed expanded tumor spreading into the peritoneum, diaphragm, kidney, stomach, intestine, liver and spleen (the spleen was enlarged in all cases). In contrast, mice treated with fragmented PAM-1 antibody showed a reduced spread of tumors and in addition there were no enlarged spleens observable. In both experiments the overall weight of tumor mass was significantly reduced by the PAM-1 antibody (Figs. 14B and 14C).

Morphological analysis of the tumors revealed that tumors from mice treated with fragmented PAM-1 antibody exhibited not only a reduced size, but also regressive changes in growth pattern like tumor-regression, infiltration and a high number of pyknotic cells (Figs. 15A and 15D). *In situ* staining of the tumors for apoptotic activity showed that the tumors from mice treated with fragmented PAM-1 antibody have a significantly higher number of tumor cells undergoing apoptosis compared to control mice (Figs. 15C and 15F). Figs. 15B and 15E show immunoperoxidase staining with an apoptosis-positive control in which all cell nucleic are stained.

Recombinant PAM-1 in vivo activity

To determine the effects of a recombinant PAM-1 IgG antibody on tumor cell growth *in vivo*, mouse-human stomach carcinoma and mouse-human pancreatic carcinoma cell systems were used. In each system C.B-17/IcrHanHsd-*scid* mice aged 6-8 weeks were injected with carcinoma cells at day 0 (n=10 per group). In the case of stomach carcinoma cells, 2.0×10^6 23132/87 cells were injected subcutaneously (s.c.) at day 0, and in the case of pancreatic carcinoma cells, 1.5×10^6 BXPC-3 cells were injected s.c. at day 0. Each mouse in the respective test and control groups was injected with 200 μg of the PAM-1 IgG antibody or the control IgG antibody (Chrompure human IgG) at day 1, 3, 5, 7, and 9.

To test the activity of the PAM-1 IgG antibody against stomach cancer, the mice were sacrificed at day 14 and the tumor weight and volume were compared between the control and test groups. As is evident from Figs. 19A and 19B, administration of the PAM-1 IgG antibody resulted in a significant reduction in stomach cancer tumor volume ($p = 0.0007$) and tumor weight ($p = 0.0036$) when compared to the control group.

Similarly, to test the activity of the PAM-1 IgG antibody against pancreatic cancer, the mice were sacrificed at day 27 and the tumor weight and volume were compared between the control and test groups. As is evident from Figs. 20A and 20B, administration of the PAM-1 IgG antibody resulted in a significant reduction in pancreatic cancer tumor volume ($p = 0.0296$) and tumor weight ($p = 0.154$) when

compared to the control group. PAM-1-induced tumor regression was also verified by staining tissue obtained from the mice injected with pancreatic cancer cells and either the PAM-1, or the control IgG antibody, for keratin or with haematoxilin-eosin. The reduction in keratin staining seen in Fig. 21C when compared with the control (Fig. 21A) indicates tumor cell death in the mice receiving the PAM-1 antibody, as does the existence of apoptotic cells in tumor tissue obtained from mice receiving the PAM-1 IgG antibody (indicated by arrows in Fig. 21D) and not in the control tissue (Fig. 21B).

Example 5
In Vivo Imaging of a Neoplasm

A patient suspected of having a neoplasm, such as stomach cancer, may be given a dose of radioiodinated PAM-1 antibody or fragment thereof, or another tumor-specific polypeptide, and radiolabeled unspecific antibody using the methods described herein. Localization of the tumor for imaging may be effected according to the procedure of Goldenberg et al. (N. Engl. J. Med., 298:1384, 1978). By I.V. an infusion of equal volumes of solutions of ^{131}I-PAM-1 antibody and Tc-99m-labeled unspecific antibody may be administered to a patient. Prior to administration of the reagents I.V., the patient is typically pre-tested for hypersensitivity to the antibody preparation (unlabeled) or to antibody of the same species as the antibody preparation. To block thyroid uptake of ^{131}I, Lugol's solution is administered orally, beginning one or more days before injection of the radioiodinated antibody, at a dose of 5 drops twice or three-times daily. Images of various body regions and views may be taken at 4, 8, and 24 hours after injection of the labeled preparations. If present, the neoplasm, e.g., a stomach adenocarcinoma, is detected by gamma camera imaging with subtraction of the Tc-99m counts from those of ^{131}I, as described for ^{131}I -labeled anti-CEA antibody and Tc- 99m-labeled human serum albumin by DeLand et al. (Cancer Res. 40:3046, 1980). At 8 hours after injection, imaging is usually clear and improves with time up to the 24 hour scans.

Example 6
Treatment of a Neoplasm Using Labeled Antibody Mixtures

A patient diagnosed with a neoplasm, for example, a patient diagnosed with a stomach adenocarcinoma, may be treated with PAM-1 antibodies or fragments thereof as follows. Lugol's solution may be administered, e.g., 7 drops 3 times daily, to the patient. Subsequently, a therapeutic dose of ^{131}I-PAM-1 antibody may be administered to the patient. For example, a ^{131}I dose of 50 mCi may be given weekly for 3 weeks, and then repeated at intervals adjusted on an individual basis, e.g., every three months, until hematological toxicity interrupts the therapy. The exact treatment regimen is generally determined by the attending physician or person supervising the treatment. The radioiodinated antibodies may be administered as slow I.V. infusions in 50 ml of sterile physiological saline. After the third injection dose, a reduction in the size of the primary tumor and metastases may be noted, particularly after the second therapy cycle, or 10 weeks after onset of therapy.

Example 7
Treatment Using Conjugated Antibodies

A patient diagnosed with a neoplasm, for example, a patient with stomach cancer that has metastasized, may be treated with solutions of ^{131}I-PAM-1, ^{10}B-PAM-1, and a Tc-99m labeled unspecific antibody. An amount of ^{131}I-labeled PAM-1 antibody (in 50 ml of sterile physiological saline) sufficient to provide 100 mCi of ^{131}I activity based on a 70 kg patient weight may be administered to the patient. This dosage is equal to 3.3 mg of an antibody having 40-80 Boron atoms and 8-16 Boron-10 atoms per antibody molecule. The neoplasm is first precisely localized using the procedure of Example 5. In addition, Lugol's solution should be continuously administered to the patient, as in the previous example. A well-collimated beam of thermal neutrons may then be focused on the defined tumor locations. Irradiation with an external neutron beam dose of 400-800 rads, delivered in a period of from 8-20 min, is effected for each tumor locus, and is optionally repeated with administration of the tumor-locating antibody, with or without the radiolabel, at intervals adjusted on an individual basis, but usually not exceeding a total dose of 3200 rads unless

simultaneous external irradiation therapy is indicated. If desired, in addition to this therapy, an anti-tumor agent, such as a chemotherapeutic agent, may also be administered to the patient.

Other Embodiments

While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure come within known or customary practice within the art to which the invention pertains and may be applied to the essential features hereinbefore set forth.

International Patent Application Nos. PCT/IB03/01335 and PCT/IB03/03487, U.S. Patent Application No.10/764,730, U.S. Patent Nos. 5,367,060, 5,641,869, 6,207,646, 6,384,018, and all other references, patents, and patent application publications cited herein are hereby incorporated by reference in their entirety.

What is claimed is:

CLAIMS

1. An isolated polypeptide that specifically binds to a neoplastic cell or a cell of a pre-cancerous lesion, but does not specifically bind to a normal cell, wherein said isolated polypeptide comprises amino acids 28-32, 51-53, and 90-100 of the sequence of SEQ ID NO:27, and wherein said normal cell is not a cell of the glomerular, fascicular zone of the adrenal gland or an epithelial cell of the collection tubes of the kidney.

2. The isolated polypeptide of claim 1, wherein said polypeptide further comprises amino acids 11-18, 36-43, and 82-104 of the sequence of SEQ ID NO:26.

3. An isolated polypeptide that specifically binds to a neoplastic cell or a cell of a pre-cancerous lesion, but does not specifically bind to a normal cell, wherein said isolated polypeptide comprises amino acids 11-15, 30-46, and 79-88 of the sequence of SEQ ID NO:2, but does not comprise the full-length sequence of SEQ ID NO:2, and wherein said normal cell is not a cell of the glomerular, fascicular zone of the adrenal gland or an epithelial cell of the collection tubes of the kidney.

4. The isolated polypeptide of claim 3, wherein said polypeptide further comprises amino acids 17-32, 48-54, and 87-95 of the sequence of SEQ ID NO:4, but does not comprise the full-length sequence of SEQ ID NO:4.

5. The isolated polypeptide of claim 1 or 3, wherein said polypeptide is capable of inducing apoptosis of said neoplastic cell or said cell of said pre-cancerous lesion, but does not induce apoptosis of said normal cell.

6. The isolated polypeptide of claim 1 or 3, wherein said neoplastic cell is selected from the group consisting of Barrett's tumors and tumors of the esophagus, stomach, intestine, rectum, liver, gallbladder, pancreas, lungs, bronchi, breast, cervix, prostate, heart, ovary, and uterus.

7. The isolated polypeptide of claim 1 or 3, wherein said pre-cancerous lesion is selected from the group consisting of dysplasia of the gastric mucosa, interstitial metaplasia of the stomach, inflammation of the gastric mucosa which is associated with the bacteria *Helicobacter pylori*, tubular and tubulovillous adenomas of the stomach, tubular adenoma of the colon, villous adenoma of the colon, dysplasia in ulcerative colitis, Barrett's dysplasia, Barrett's metaplasia of the esophagus, cervical intraepithelial neoplasia I, cervical intraepithelial neoplasia II, cervical intraepithelial neoplasia III, squamous epithelial metaplasia, squamous epithelial dysplasia of the bronchus, low grade and high grade prostate intraepithelial neoplasia (PIN), breast ductal carcinoma in situ (D-CIS), and breast lobular carcinoma in situ (L-CIS).

8. The isolated polypeptide of claim 1 or 3, wherein said polypeptide is a functional fragment of an antibody selected from the group consisting of V_L, V_H, F_V, F_C, Fab, Fab', and $F(ab')_2$.

9. The isolated polypeptide of claim 1 or 3, wherein said polypeptide specifically binds to a polypeptide comprising the sequence of SEQ ID NO:6.

10. An isolated nucleic acid molecule comprising nucleic acids 31-54, 106-129, and 244-312 of the sequence of SEQ ID NO:28, and/or 82-96, 151-159, and or 268-300 of the sequence of SEQ ID NO:29.

11. An isolated nucleic acid molecule comprising nucleic acids 31-45, 88-138, and 235-264 of the sequence of SEQ ID NO:1, and/or nucleic acids 49-96, 142-162, and 259-285 of the sequence of SEQ ID NO:3, wherein said nucleic acid molecule does not comprise the full-length sequence of SEQ ID NO:1 or SEQ ID NO:3.

12. An isolated nucleic acid molecule comprising the sequence of SEQ ID NO:5.

13. A vector comprising the nucleic acid sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:28, or SEQ ID NO:29.

14. An isolated cell comprising the vector of claim 13.

15. An isolated cell that expresses the polypeptide of claim 1 or 3.

16. The isolated cell of claim 15, wherein said isolated cell is a mammalian cell.

17. The isolated cell of claim 16, wherein said mammalian cell is a human cell.

18. A method of producing the purified polypeptide of claim 1, said method comprising contacting a cell with a vector comprising SEQ ID NO:29 and isolating the polypeptide expressed by said vector.

19. The method of claim 18, wherein said vector further comprises the sequence of SEQ ID NO:28.

20. A method of diagnosing a neoplasm or a pre-cancerous lesion in a mammal, said method comprising the steps of, (a) contacting a cell or tissue sample derived from said mammal with the purified polypeptide of claim 1 or 3, and (b) detecting whether said purified polypeptide specifically binds to said cell or tissue sample, wherein specific binding of said purified polypeptide to said cell or tissue sample is indicative of said mammal having a neoplasm or pre-cancerous lesion.

21. The method of claim 20, wherein said cell or tissue sample is derived from a tissue selected from the group consisting of Barrett's tumors, tumors of the esophagus, stomach, intestine, rectum, liver, gallbladder, pancreas, lungs, bronchi, breast, cervix, prostate, heart, ovary, and uterus, dysplasia of the gastric mucosa, interstitial metaplasia of the stomach, inflammation of the gastric mucosa which is associated with the bacteria *Helicobacter pylori*, tubular and tubulovillous adenomas of the stomach, tubular adenoma of the colon, villous adenoma of the colon, dysplasia in ulcerative colitis, Barrett's dysplasia, Barrett's metaplasia of the esophagus, cervical intraepithelial neoplasia I, cervical intraepithelial neoplasia II, cervical intraepithelial neoplasia III, squamous epithelial metaplasia, squamous epithelial dysplasia of the bronchus, low grade and high grade prostate intraepithelial neoplasia (PIN), breast ductal carcinoma in situ (D-CIS), and breast lobular carcinoma in situ (L-CIS).

22. The method of claim 20, wherein said polypeptide is conjugated to a detectable agent selected from the group consisting of a radionuclide, a fluorescent marker, an enzyme, a cytotoxin, a cytokine, and a growth inhibitor.

23. The method of claim 22, wherein said detectable agent is capable of inducing apoptosis of said cell.

24. The method of claim 20, wherein said polypeptide is conjugated to a protein purification tag.

25. The method of claim 24, wherein said protein purification tag is cleavable.

26. The method of claim 20, wherein said mammal is a human.

27. The method of claim 20, wherein said polypeptide is an antibody.

28. The method of claim 27, wherein said polypeptide is murine antibody 58/47-69.

29. A method of treating a proliferative disorder in a mammal, said method comprising the step of contacting a cell with the purified polypeptide of claim 1 or 3, wherein binding of said purified polypeptide to said cell results in the induction of apoptosis of said cell.

30. The method of claim 29, wherein said mammal is a human.

31. The method of claim 29, wherein said polypeptide is an antibody.

32. The method of claim 31, wherein said antibody is murine antibody 58/47-

33. The method of claim 31, wherein said antibody is a humanized antibody.

34. The method of claim 29, wherein said polypeptide is conjugated to a detectable agent selected from the group consisting of a radionuclide, a fluorescent marker, an enzyme, a cytotoxin, a cytokine, and a growth inhibitor.

35. The method of claim 34, wherein said polypeptide is conjugated to a protein purification tag.

36. The method of claim 35, wherein said protein purification tag is cleavable.

37. A pharmaceutical composition comprising the isolated polypeptide of claim 1 in a pharmaceutically acceptable carrier.

38. A diagnostic agent comprising the isolated polypeptide of claim 1 or 3.

39. An isolated polypeptide, wherein said polypeptide comprises an amino acid sequence consisting of amino acids 469-518 of SEQ ID NO:6 or amino acids 739-748 of SEQ ID NO:6, and wherein said polypeptide does not comprise the full-length sequence of SEQ ID NO:6.

40. The isolated polypeptide of claim 39, wherein said polypeptide comprises an amino acid sequence consisting of amino acids 469-518 of SEQ ID NO:6.

41. The isolated polypeptide of claim 39, wherein said polypeptide comprises an amino acid sequence consisting of amino acids 739-748 of SEQ ID NO:6.

42. The isolated polypeptide of claim 39, wherein said polypeptide is at least 95% pure.

43. The isolated polypeptide of claim 39, wherein said polypeptide is specifically bound by murine antibody 58/47-69.

44. The isolated polypeptide of claim 39, wherein said polypeptide comprises a tumor-specific glycostructure.

45. The isolated polypeptide of claim 39, wherein said polypeptide is expressed by a pre-cancerous lesion selected from the group consisting of dysplasia of the gastric mucosa, interstitial metaplasia of the stomach, inflammation of the gastric mucosa which is associated with the bacteria *Helicobacter pylori*, tubular and tubulovillous adenomas of the stomach, tubular adenoma of the colon, villous adenoma of the colon, dysplasia in ulcerative colitis, Barrett's dysplasia, Barrett's metaplasia of the esophagus, cervical intraepithelial neoplasia I, cervical intraepithelial neoplasia II, cervical intraepithelial neoplasia III, squamous epithelial metaplasia, squamous epithelial dysplasia of the bronchus, low grade and high grade prostate intraepithelial neoplasia (PIN), breast ductal carcinoma in situ (D-CIS), and breast lobular carcinoma in situ (L-CIS), and not by normal cells of the same tissue type.

46. The isolated polypeptide of claim 39, wherein said polypeptide is expressed by a tumor selected from the group consisting of Barrett's tumors and tumors of the esophagus, stomach, intestine, rectum, liver, gallbladder, pancreas, lungs, bronchi, breast, cervix, prostate, heart, ovary, and uterus, and not by a normal cell of the same tissue type.

47. A diagnostic agent comprising the isolated polypeptide of claim 39.

48. A method of inducing a tumor-specific immune response in a mammal, said method comprising the step of contacting said mammal with an isolated polypeptide comprising the sequence of SEQ ID NO:6, or a fragment comprising amino acids 469-518 of SEQ ID NO:6 or amino acids 739-748 of SEQ ID NO:6, wherein said contacting induces a tumor-specific immune response in said mammal.

49. The method of claim 48, wherein said tumor-specific immune response comprises the production of an antibody that induces apoptosis of a cell which is specifically bound by said antibody.

50. The method of claim 48, wherein said fragment comprises amino acids 469-518 of SEQ ID NO:6 and amino acids 739-748 of SEQ ID NO:6 and does not comprise the full-length sequence of SEQ ID NO:6.

51. A method of producing an isolated polypeptide comprising the sequence of SEQ ID NO:6 or a fragment thereof comprising amino acids 469-518 of SEQ ID NO:6 or amino acids 739-748 of SEQ ID NO:6, said method comprising the steps of (a) contacting a cell with a vector comprising a nucleic acid sequence that is substantially identical to SEQ ID NO:5 and (b) isolating the polypeptide expressed by said cell.

52. A method of identifying a candidate therapeutic compound, said method comprising the steps of (a) contacting a cell expressing a polypeptide comprising the amino acid sequence of SEQ ID NO:6, or a fragment thereof comprising amino acids 469-518 of SEQ ID NO:6 or amino acids 739-748 of SEQ ID NO:6, with a test compound and (b) determining whether said test compound induces apoptosis of said cell and not of a control cell contacted with said test compound, wherein a test compound that induces apoptosis of said cell and not of said control cell is a candidate therapeutic compound.

53. The method of claim 52, wherein said fragment comprises amino acids 469-518 of SEQ ID NO:6 and amino acids 739-748 of SEQ ID NO:6 and does not comprise the full-length sequence of SEQ ID NO:6.

54. The method of claim 52, wherein said cell is adenocarcinoma cell line 23132 (DSMZ Accession No. DSM ACC 201).

55. A method of producing an antibody that specifically binds to a neoplastic cell, said method comprising (a) administering the purified polypeptide of claim 39 to a mammal and (b) isolating from said mammal, an antibody that specifically binds to said polypeptide.

56. The method of claim 55, wherein said polypeptide is purified from adenocarcinoma cell line 23132 (DSMZ Accession No. DSM ACC 201).

57. The method of claim 55, wherein said method further comprises isolating a cell expressing said antibody from said mammal.

Fig. 1

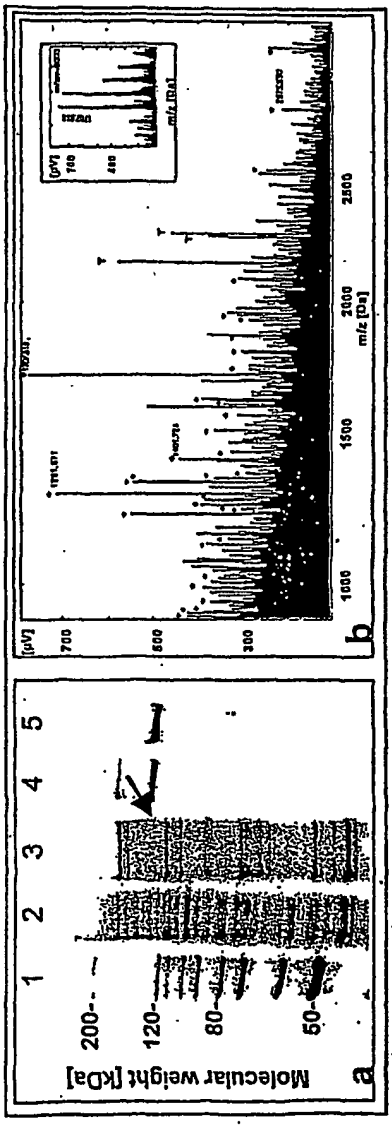


Fig. 2

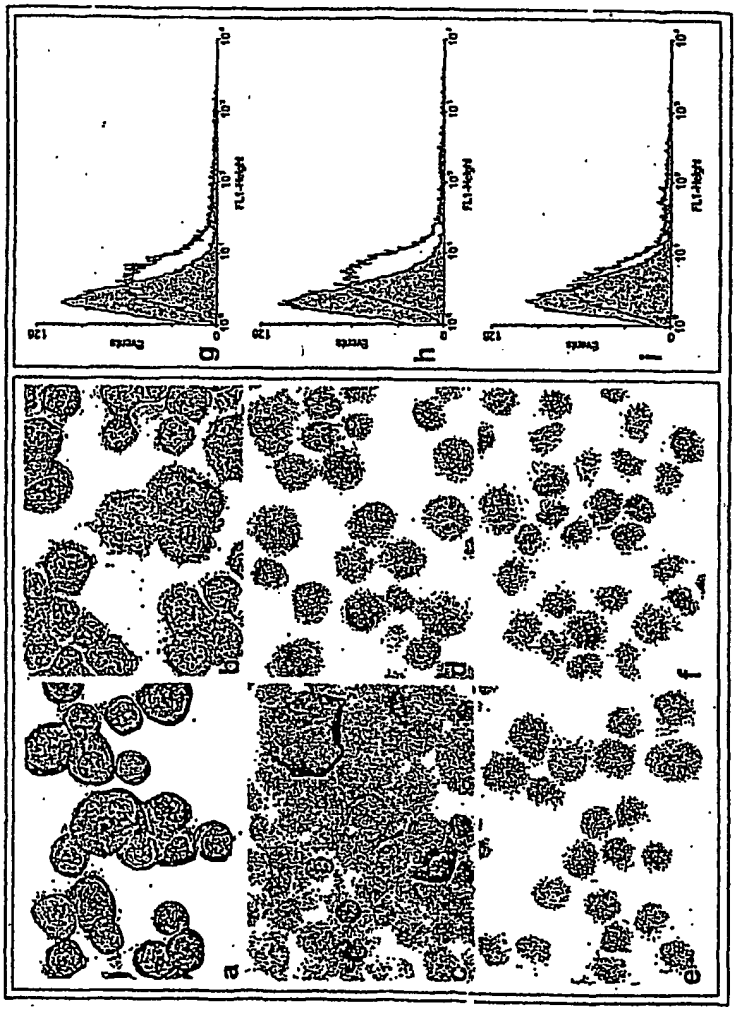


Fig. 3

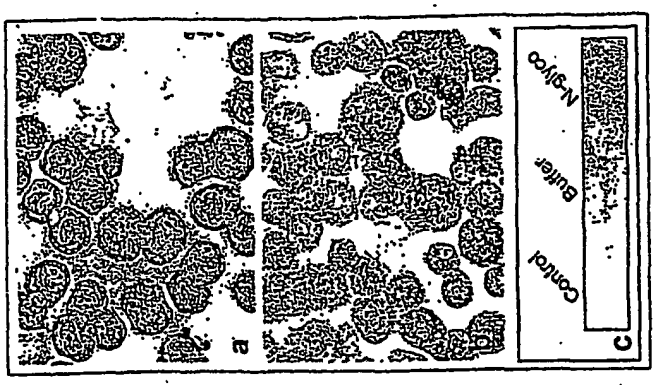


Fig. 4

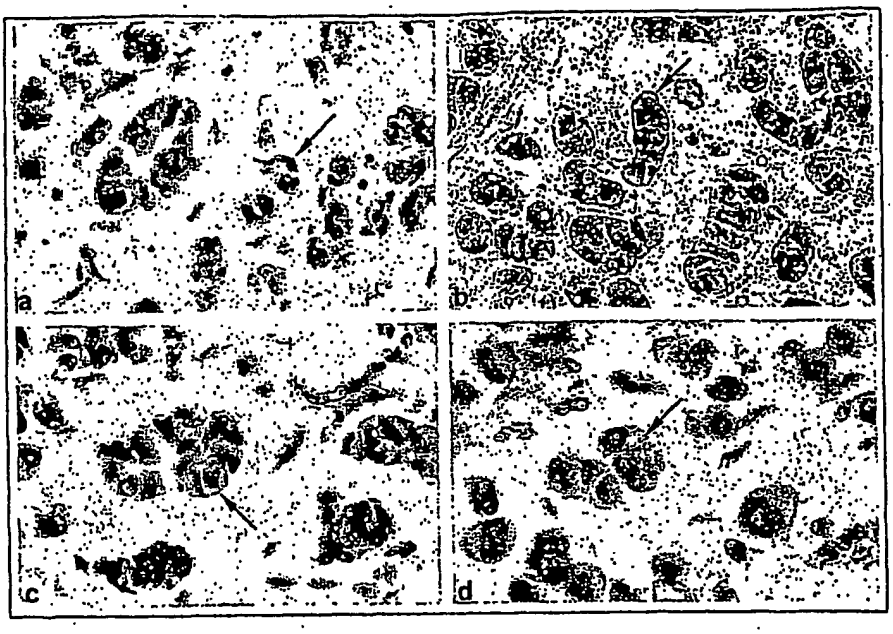


Fig. 5

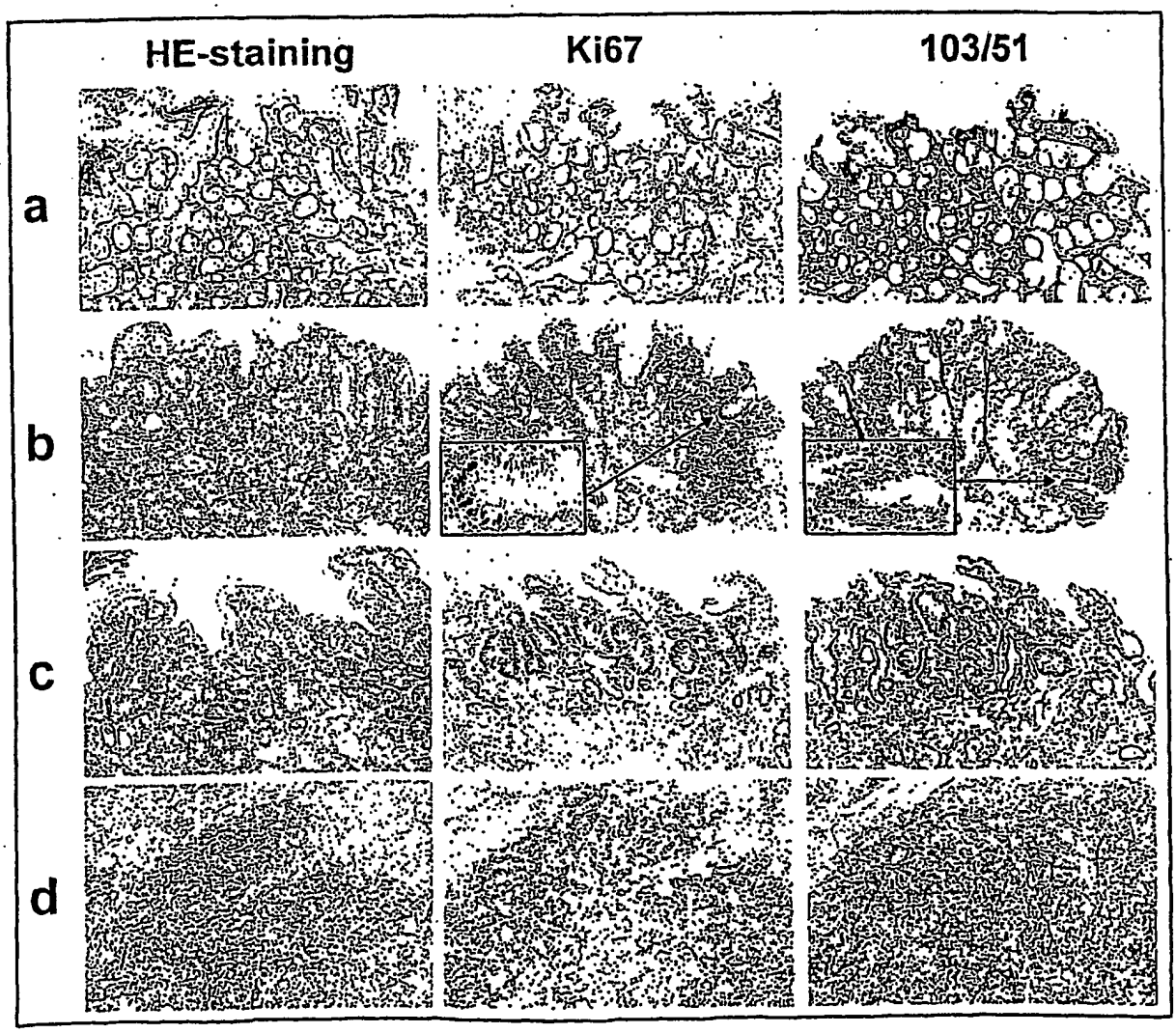


Fig. 6

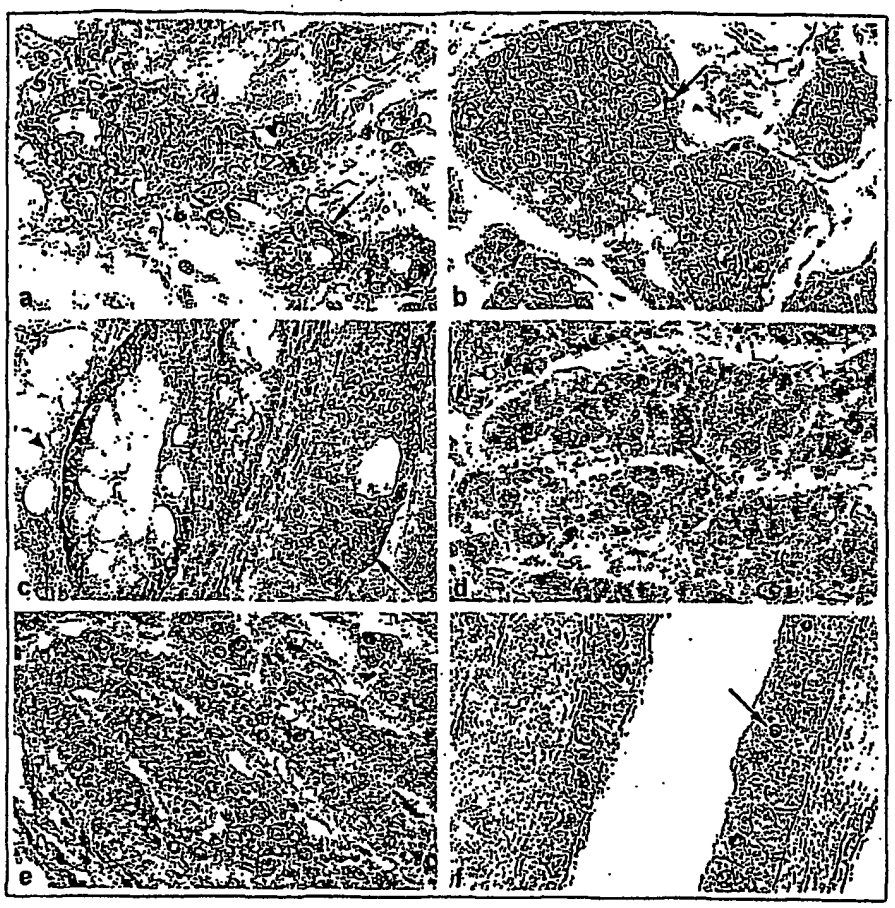
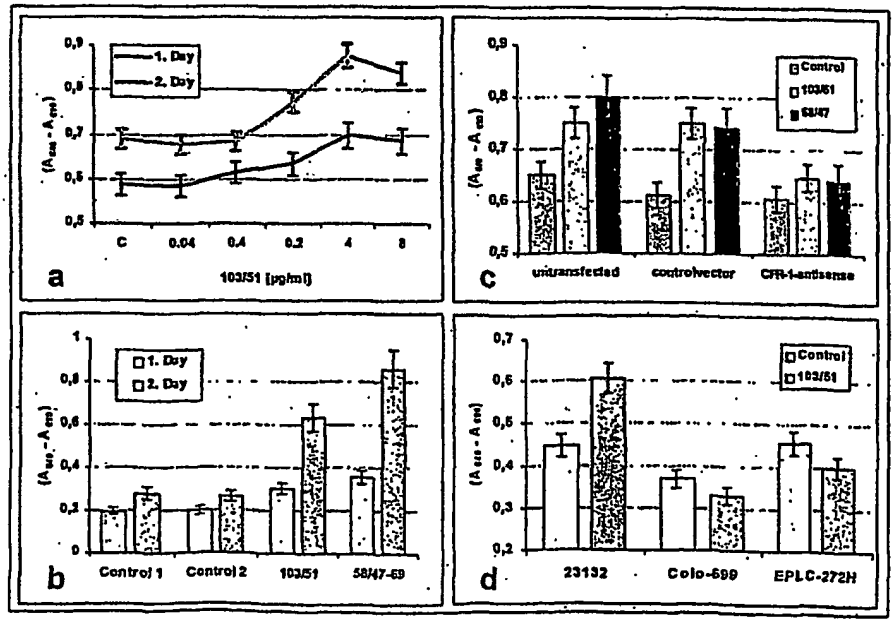

a
b
c
d
e
f

Fig. 7


1. Day
2. Day
C
0.04
0.4
0.2
4
8
103/51 [pg/ml]
a
Control
103/51
58/47
untransfected
controlvector
CFR-1-antisense
c
Control 1
Control 2
103/51
58/47-69
b
23132
Colo-699
EPLC-272H
d

```
tcc tgc aag gct tct ggc tac acc ttc act gac tac tat ata aac     45
Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asp Tyr Tyr Ile Asn
 1               5                  10                  15

tgg gtg aag cag agg act gga cag ggc ctt gag tgg att gga gag     90
Trp Val Lys Gln Arg Thr Gly Gln Gly Leu Glu Trp Ile Gly Glu
            20                  25                  30

att tat cct gga agt ggt aat act tac tac aat gag aag ttc aag    135
Ile Tyr Pro Gly Ser Gly Asn Thr Tyr Tyr Asn Glu Lys Phe Lys
        35                  40                  45

ggc aag gcc aca ctg act gca gac aaa tcc tcc agc aca gcc tac    180
Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser Thr Ala Tyr
    50                  55                  60

atg cag ctc agc agc ctg aca tct gag gac tct gca gtc tat ttc    225
Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Phe
65                  70                  75

tgt gca aga tcg gga tta cga ccc tat gct atg gac tac tgg ggt    270
Cys Ala Arg Ser Gly Leu Arg Pro Tyr Ala Met Asp Tyr Trp Gly
            80                  85                  90

caa gga acc tca gtc acc
Gln Gly Thr Ser Val Thr
        95
```

Fig. 8A

Nucleotide sequence of the variable region of the heavy chain (VH) of antibody NM58-49/69

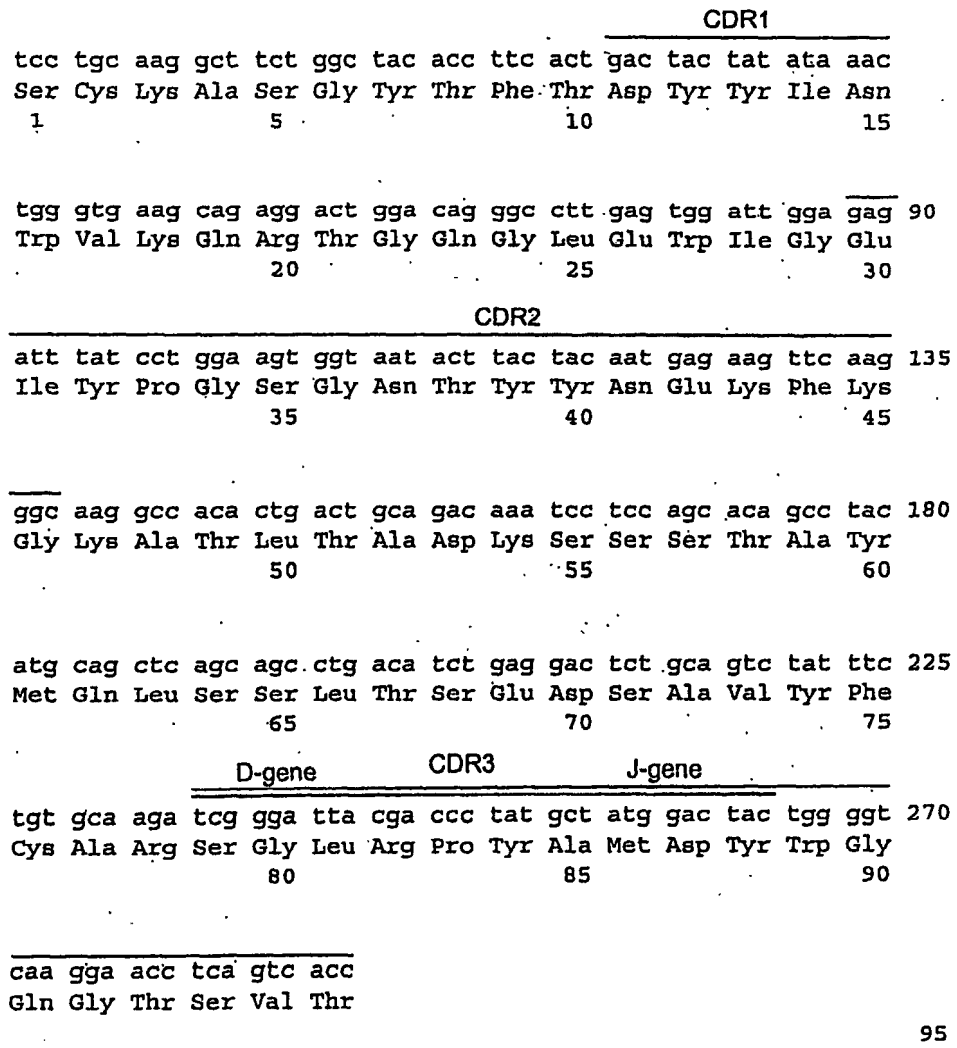


Fig. 8B

```
cca ctc tcc ctg cct gtc agt ctt gga gat caa gcc tcc atc tct     45
Pro Leu Ser Leu Pro Val Ser Leu Gly Asp Gln Ala Ser Ile Ser
 1               5                  10                  15

tgc aga tct agt cag agc att gta cat agt aat gga aac acc tat     90
Cys Arg Ser Ser Gln Ser Ile Val His Ser Asn Gly Asn Thr Tyr
                20                  25                  30

tta gaa tgg tac ctg cag aaa cca ggc cag tct cca aag ctc ctg     135
Leu Glu Trp Tyr Leu Gln Lys Pro Gly Gln Ser Pro Lys Leu Leu
                35                  40                  45

atc tac aaa gtt tcc aac cga ttt tct ggg gtc cca gac agg ttc     180
Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro Asp Arg Phe
                50                  55                  60

agt ggc agt gga tca ggg aca gat ttc aca ctc aag atc agc aga     225
Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile Ser Arg
                65                  70                  75

gtg gag gct gag gat ctg gga gtt tat tac tgc ttt caa ggt tca     270
Val Glu Ala Glu Asp Leu Gly Val Tyr Tyr Cys Phe Gln Gly Ser
                80                  85                  90

cat gtt ccg tac acg ttc gga ggg ggg acc aag ctg gaa ata aaa     315
His Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
                95                  100                 105
```

Fig. 9A

Nucleotide sequence of the variable region of the light chain (VL) of antibody NM58-49/69

```
cca ctc tcc ctg cct gtc agt ctt gga gat caa gcc tcc atc tct 45
Pro Leu Ser Leu Pro Val Ser Leu Gly Asp Gln Ala Ser Ile Ser
 1               5                  10                  15

                                CDR1
                  ____________________________________________
tgc aga tct agt cag agc att gta cat agt aat gga aac acc tat 90
Cys Arg Ser Ser Gln Ser Ile Val His Ser Asn Gly Asn Thr Tyr
                20                  25                  30

_____
tta gaa tgg tac ctg cag aaa cca ggc cag tct cca aag ctc ctg 135
Leu Glu Trp Tyr Leu Gln Lys Pro Gly Gln Ser Pro Lys Leu Leu
                35                  40                  45

                     CDR2
                  ____________________
atc tac aaa gtt tcc aac cga ttt tct ggg gtc cca gac agg ttc 180
Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro Asp Arg Phe
                50                  55                  60

agt ggc agt gga tca ggg aca gat ttc aca ctc aag atc agc aga 225
Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile Ser Arg
                65                  70                  75

                                                    CDR3
                                                  ________
gtg gag gct gag gat ctg gga gtt tat tac tgc ttt caa ggt tca 270
Val Glu Ala Glu Asp Leu Gly Val Tyr Tyr Cys Phe Gln Gly Ser
                80                  85                  90

_____________________
cat gtt ccg tac acg ttc gga ggg ggg acc aag ctg gaa ata aaa 315
His Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
                95                 100                 105
```

Fig. 9B

```
gat gtg agg gag cct gaa aat gaa att tct tca gac tgc aat cat          45
Asp Val Arg Glu Pro Glu Asn Glu Ile Ser Ser Asp Cys Asn His
                5                  10                  15

ttg ttg tgg aat tat aag ctg aac cta act aca gat ccc aaa ttt          90
Leu Leu Trp Asn Tyr Lys Leu Asn Leu Thr Thr Asp Pro Lys Phe
            20                  25                  30

gaa tct gtg gcc aga gag gtt tgc aaa tct act ata aca gag att         135
Glu Ser Val Ala Arg Glu Val Cys Lys Ser Thr Ile Thr Glu Ile
        35                  40                  45

gaa gaa tgt gct gat gaa ccg gtt gga aaa ggt tac atg gtt tcc         180
Glu Glu Cys Ala Asp Glu Pro Val Gly Lys Gly Tyr Met Val Ser
    50                  55                  60

tgc ttg gtg gat cac cga ggc aac atc act gag tat cag tgt cac         225
Cys Leu Val Asp His Arg Gly Asn Ile Thr Glu Tyr Gln Cys His
65                  70                  75

cag tac att acc aag atg acg gcc atc att ttt agt gat tac cgt         270
Gln Tyr Ile Thr Lys Met Thr Ala Ile Ile Phe Ser Asp Tyr Arg
            80                  85                  90

tta atc tgt ggc ttc atg gat gac tgc aaa aat gac atc aac att         315
Leu Ile Cys Gly Phe Met Asp Asp Cys Lys Asn Asp Ile Asn Ile
        95                  100                 105

ctg aaa tgt ggc agt att cgg ctt gga gaa aag gat gca cat tca         360
Leu Lys Cys Gly Ser Ile Arg Leu Gly Glu Lys Asp Ala His Ser
    110                 115                 120

caa ggt gag gtg gta tca tgc ttg gag aaa ggc ctg gtg aaa gaa         405
Gln Gly Glu Val Val Ser Cys Leu Glu Lys Gly Leu Val Lys Glu
125                 130                 135

gca gaa gaa aga gaa ccc aag att caa gtt tct gaa ctc tgc aag         450
Ala Glu Glu Arg Glu Pro Lys Ile Gln Val Ser Glu Leu Cys Lys
            140                 145                 150

aaa gcc att ctc cgg gtg gct gag ctg tca tcg gat gac ttt cac         495
Lys Ala Ile Leu Arg Val Ala Glu Leu Ser Ser Asp Asp Phe His
        155                 160                 165

tta gac cgg cat tta tat ttt gct tgc cga gat gat cgg gag cgt         540
Leu Asp Arg His Leu Tyr Phe Ala Cys Arg Asp Asp Arg Glu Arg
    170                 175                 180

ttt tgt gaa aat aca caa gct ggt gag ggc aga gtg tat aag tgc         585
Phe Cys Glu Asn Thr Gln Ala Gly Glu Gly Arg Val Tyr Lys Cys
185                 190                 195

ctc ttt aac cat aaa ttt gaa gaa tcc atg agt gaa aag tgt cga         630
Leu Phe Asn His Lys Phe Glu Glu Ser Met Ser Glu Lys Cys Arg
            200                 205                 210
```

Fig. 10-1

```
gaa gca ctt aca acc cgc caa aag ctg att gcc cag gat tat aaa        675
Glu Ala Leu Thr Thr Arg Gln Lys Leu Ile Ala Gln Asp Tyr Lys
                215                 220                 225

gtc agt tat tca ttg gcc aaa tcc tgt aaa agt gac ttg aag aaa        720
Val Ser Tyr Ser Leu Ala Lys Ser Cys Lys Ser Asp Leu Lys Lys
                230                 235                 240

tac cgg tgc aat gtg gaa aac ctt ccg cga tcg cgt gaa gcc agg        765
Tyr Arg Cys Asn Val Glu Asn Leu Pro Arg Ser Arg Glu Ala Arg
                245                 250                 255

ctc tcc tac ttg tta atg tgc ctg gag tca gct gta cac aga ggg        810
Leu Ser Tyr Leu Leu Met Cys Leu Glu Ser Ala Val His Arg Gly
                260                 265                 270

cga caa gtc agc agt gag tgc cag ggg gag atg ctg gat tac cga        855
Arg Gln Val Ser Ser Glu Cys Gln Gly Glu Met Leu Asp Tyr Arg
                275                 280                 285

cgc atg ttg atg gaa gac ttt tct ctg agc cct gag atc atc cta        900
Arg Met Leu Met Glu Asp Phe Ser Leu Ser Pro Glu Ile Ile Leu
                290                 295                 300

agc tgt cgg ggg gag att gaa cac cat tgt tcc gga tta cat cga        945
Ser Cys Arg Gly Glu Ile Glu His His Cys Ser Gly Leu His Arg
                305                 310                 315

aaa ggg cgg acc cta cac tgt ctg atg aaa gta gtt cga ggg gag        990
Lys Gly Arg Thr Leu His Cys Leu Met Lys Val Val Arg Gly Glu
                320                 325                 330

aag ggg aac ctt gga atg aac tgc cag cag gcg ctt caa aca ctg       1035
Lys Gly Asn Leu Gly Met Asn Cys Gln Gln Ala Leu Gln Thr Leu
                335                 340                 345

att cag gag act gac cct ggt gca gat tac cgc att gat cga gct       1080
Ile Gln Glu Thr Asp Pro Gly Ala Asp Tyr Arg Ile Asp Arg Ala
                350                 355                 360

ttg aat gaa gct tgt gaa tct gta atc cag aca gcc tgc aaa cat       1125
Leu Asn Glu Ala Cys Glu Ser Val Ile Gln Thr Ala Cys Lys His
                365                 370                 375

ata aga tct gga gac cca atg atc ttg tcg tgc ctg atg gaa cat       1170
Ile Arg Ser Gly Asp Pro Met Ile Leu Ser Cys Leu Met Glu His
                380                 385                 390

tta tac aca gag aag atg gta gaa gac tgt gaa cac cgt ctc tta       1215
Leu Tyr Thr Glu Lys Met Val Glu Asp Cys Glu His Arg Leu Leu
            395                 400                 405

gag ctg cag tat ttc atc tcc cgg gat tgg aag ctg gac cct gtc       1260
Glu Leu Gln Tyr Phe Ile Ser Arg Asp Trp Lys Leu Asp Pro Val
                410                 415                 420

ctg tac cgc aag tgc cag gga gac gct tct cgt ctt tgc cac acc       1305
Leu Tyr Arg Lys Cys Gln Gly Asp Ala Ser Arg Leu Cys His Thr
                425                 430                 435

cac ggt tgg aat gag acc agc gaa ttt atg cct cag gga gct gtg       1350
His Gly Trp Asn Glu Thr Ser Glu Phe Met Pro Gln Gly Ala Val
                440                 445                 450

ttc tct tgt tta tac aga cac gcc tac cgc act gag gaa cag gga       1395
Phe Ser Cys Leu Tyr Arg His Ala Tyr Arg Thr Glu Glu Gln Gly
                455                 460                 465
```

Fig. 10-2

```
agg agg ctc tca cgg gag tgc cga gct gaa gtc caa agg atc cta        1440
Arg Arg Leu Ser Arg Glu Cys Arg Ala Glu Val Gln Arg Ile Leu
                470                 475                 480

cac cag cgt gcc atg gat gtc aag ctg gat cct gcc ctc cag gat        1485
His Gln Arg Ala Met Asp Val Lys Leu Asp Pro Ala Leu Gln Asp
                485                 490                 495

aag tgc ctg att gat ctg gga aaa tgg tgc agt gag aaa aca gag        1530
Lys Cys Leu Ile Asp Leu Gly Lys Trp Cys Ser Glu Lys Thr Glu
                500                 505                 510

act gga cag aag ctg gag tgc ctt cag gac cat ctg gat gac tta        1575
Thr Gly Gln Lys Leu Glu Cys Leu Gln Asp His Leu Asp Asp Leu
                515                 520                 525

gtg gtg gag tgt aga gat ata gtt ggc aac ctc act gag tta gaa        1620
Val Val Glu Cys Arg Asp Ile Val Gly Asn Leu Thr Glu Leu Glu
                530                 535                 540

tca gag gat att caa ata gaa gcc ttg ctg atg aga gcc tgt gag        1665
Ser Glu Asp Ile Gln Ile Glu Ala Leu Leu Met Arg Ala Cys Glu
                545                 550                 555

ccc ata att cag aac ttc tgc cac gat gtg gca gat aac cag ata        1710
Pro Ile Ile Gln Asn Phe Cys His Asp Val Ala Asp Asn Gln Ile
                560                 565                 570

gac tcc ggg gac ctg atg gag tgt ctg ata cag aac aaa cac cag        1755
Asp Ser Gly Asp Leu Met Glu Cys Leu Ile Gln Asn Lys His Gln
                575                 580                 585

aag gac atg aac gag aag tgt gcc atc gga gtt acc cac ttc cag        1800
Lys Asp Met Asn Glu Lys Cys Ala Ile Gly Val Thr His Phe Gln
                590                 595                 600

ctg gtg cag atg aag gat ttt cgg ttt tct tac aag ttt aaa atg        1845
Leu Val Gln Met Lys Asp Phe Arg Phe Ser Tyr Lys Phe Lys Met
                605                 610                 615

gcc tgc aag gag gac gtg ttg aag ctt tgc cca aac ata aaa aag        1890
Ala Cys Lys Glu Asp Val Leu Lys Leu Cys Pro Asn Ile Lys Lys
                620                 625                 630

aag gtg gac gtg gtg atc tgc ctg agc acg acc gtg cgc aat gac        1935
Lys Val Asp Val Val Ile Cys Leu Ser Thr Thr Val Arg Asn Asp
                635                 640                 645

act ctg cag gaa gcc aag gag cac agg gtg tcc ctg aag tgc cgc        1980
Thr Leu Gln Glu Ala Lys Glu His Arg Val Ser Leu Lys Cys Arg
                650                 655                 660

agg cag ctc cgt gtg gag gag ctg gag atg acg gag gac atc cgc        2025
Arg Gln Leu Arg Val Glu Glu Leu Glu Met Thr Glu Asp Ile Arg
                665                 670                 675

ttg gag cca gat cta tac gaa gcc tgc aag agt gac atc aaa aac        2070
Leu Glu Pro Asp Leu Tyr Glu Ala Cys Lys Ser Asp Ile Lys Asn
                680                 685                 690

ttc tgt tcc gct gtg caa tat ggc aac gct cag att atc gaa tgt        2115
Phe Cys Ser Ala Val Gln Tyr Gly Asn Ala Gln Ile Ile Glu Cys
                695                 700                 705

ctg aaa gaa aac aag aag cag cta agc acc cgc tgc cac caa aaa        2160
Leu Lys Glu Asn Lys Lys Gln Leu Ser Thr Arg Cys His Gln Lys
                710                 715                 720
```

Fig. 10-3

```
gta ttt aag ctg cag gag aca gag atg atg gac cca gag cta gac        2205
Val Phe Lys Leu Gln Glu Thr Glu Met Met Asp Pro Glu Leu Asp
                725                 730                 735

tac acc ctc atg agg gtc tgc aag cag atg ata aag aag ttc tgt        2250
Tyr Thr Leu Met Arg Val Cys Lys Gln Met Ile Lys Lys Phe Cys
                740                 745                 750

ccg gaa gca gat tct aaa acc atg ttg cag tgc ttg aag caa aat        2295
Pro Glu Ala Asp Ser Lys Thr Met Leu Gln Cys Leu Lys Gln Asn
                755                 760                 765

aaa aac agt gaa ttg atg gat ccc aaa tgc aaa cag atg ata acc        2340
Lys Asn Ser Glu Leu Met Asp Pro Lys Cys Lys Gln Met Ile Thr
                770                 775                 780

aag cgc cag atc acc cag aac aca gat tac cgc tta aac ccc atg        2385
Lys Arg Gln Ile Thr Gln Asn Thr Asp Tyr Arg Leu Asn Pro Met
                785                 790                 795

tta aga aaa gcc tgt aaa gct gac att cct aaa ttc tgt cac ggt        2430
Leu Arg Lys Ala Cys Lys Ala Asp Ile Pro Lys Phe Cys His Gly
                800                 805                 810

atc ctg act aag gcc aag gat gat tca gaa tta gaa gga caa gtc        2475
Ile Leu Thr Lys Ala Lys Asp Asp Ser Glu Leu Glu Gly Gln Val
                815                 820                 825

atc tct tgc ctg aag ctg aga tat gct gac cag cgc ctg tct tca        2520
Ile Ser Cys Leu Lys Leu Arg Tyr Ala Asp Gln Arg Leu Ser Ser
                830                 835                 840

gac tgt gaa gac cag atc cga atc att atc cag gag tcc gcc ctg        2565
Asp Cys Glu Asp Gln Ile Arg Ile Ile Ile Gln Glu Ser Ala Leu
                845                 850                 855

gac tac cgc ctg gat cct cag ctc cag ctg cac tgc tca gac gag        2610
Asp Tyr Arg Leu Asp Pro Gln Leu Gln Leu His Cys Ser Asp Glu
                860                 865                 870

atc tcc agt cta tgt gct gaa gaa gca gca gcc caa gag cag aca        2655
Ile Ser Ser Leu Cys Ala Glu Glu Ala Ala Ala Gln Glu Gln Thr
                875                 880                 885

ggt cag gtg gag gag tgc ctc aag gtc aac ctg ctc aag atc aaa        2700
Gly Gln Val Glu Glu Cys Leu Lys Val Asn Leu Leu Lys Ile Lys
                890                 895                 900

aca gaa ttg tgt aaa aag gaa gtg cta aac atg ctg aag gaa agc        2745
Thr Glu Leu Cys Lys Lys Glu Val Leu Asn Met Leu Lys Glu Ser
                905                 910                 915

aaa gca gac atc ttt gtt gac ccg gta ctt cat act gct tgt gcc        2790
Lys Ala Asp Ile Phe Val Asp Pro Val Leu His Thr Ala Cys Ala
                920                 925                 930

ctg gac att aaa cac cac tgc gca gcc atc acc cct ggc cgc ggg        2835
Leu Asp Ile Lys His His Cys Ala Ala Ile Thr Pro Gly Arg Gly
                935                 940                 945

cgt caa atg tcc tgt ctc atg gaa gca ctg gag gat aag cgg gtg        2880
Arg Gln Met Ser Cys Leu Met Glu Ala Leu Glu Asp Lys Arg Val
                950                 955                 960

agg tta cag ccc gag tgc aaa aag cgc ctc aat gac cgg att gag        2925
Arg Leu Gln Pro Glu Cys Lys Lys Arg Leu Asn Asp Arg Ile Glu
                965                 970                 975
```

Fig. 10-4

```
atg tgg agt tac gca gca aag gtg gcc cca gca gat ggc ttc tct          2970
Met Trp Ser Tyr Ala Ala Lys Val Ala Pro Ala Asp Gly Phe Ser
                980                 985                 990

gat ctt gcc atg caa gta atg acg tct cca tct aag aac tac att          3015
Asp Leu Ala Met Gln Val Met Thr Ser Pro Ser Lys Asn Tyr Ile
                995                1000                1005

ctc tct gtg atc agt ggg agc atc tgt ata ttg ttc ctg att ggc          3060
Leu Ser Val Ile Ser Gly Ser Ile Cys Ile Leu Phe Leu Ile Gly
               1010                1015                1020

ctg atg tgt gga cgg atc acc aag cga gtg aca cga gag ctc aag          3105
Leu Met Cys Gly Arg Ile Thr Lys Arg Val Thr Arg Glu Leu LysA
               1025                1030                1035

gac agg tag
Asp Arg ***
       1038
```

Fig. 10-5

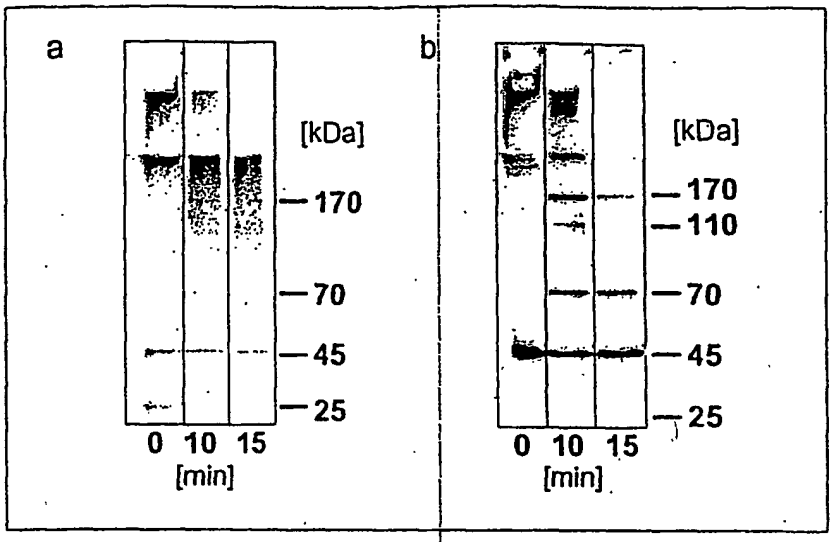


Fig. 11

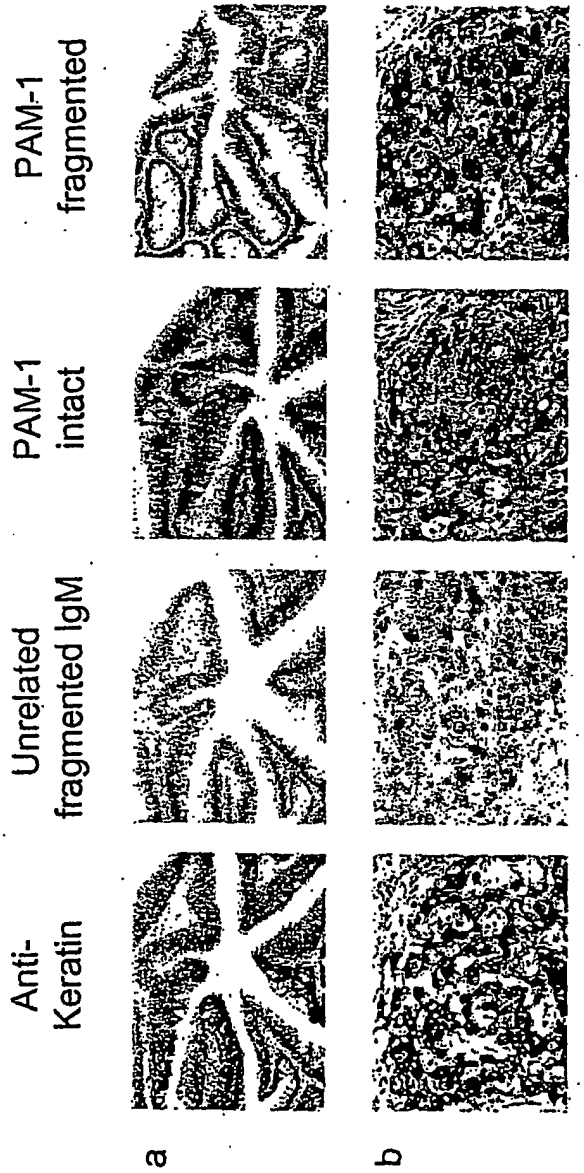

Anti-Keratin
Unrelated fragmented IgM
PAM-1 intact
PAM-1 fragmented
a
b

Fig. 12

Fig. 13

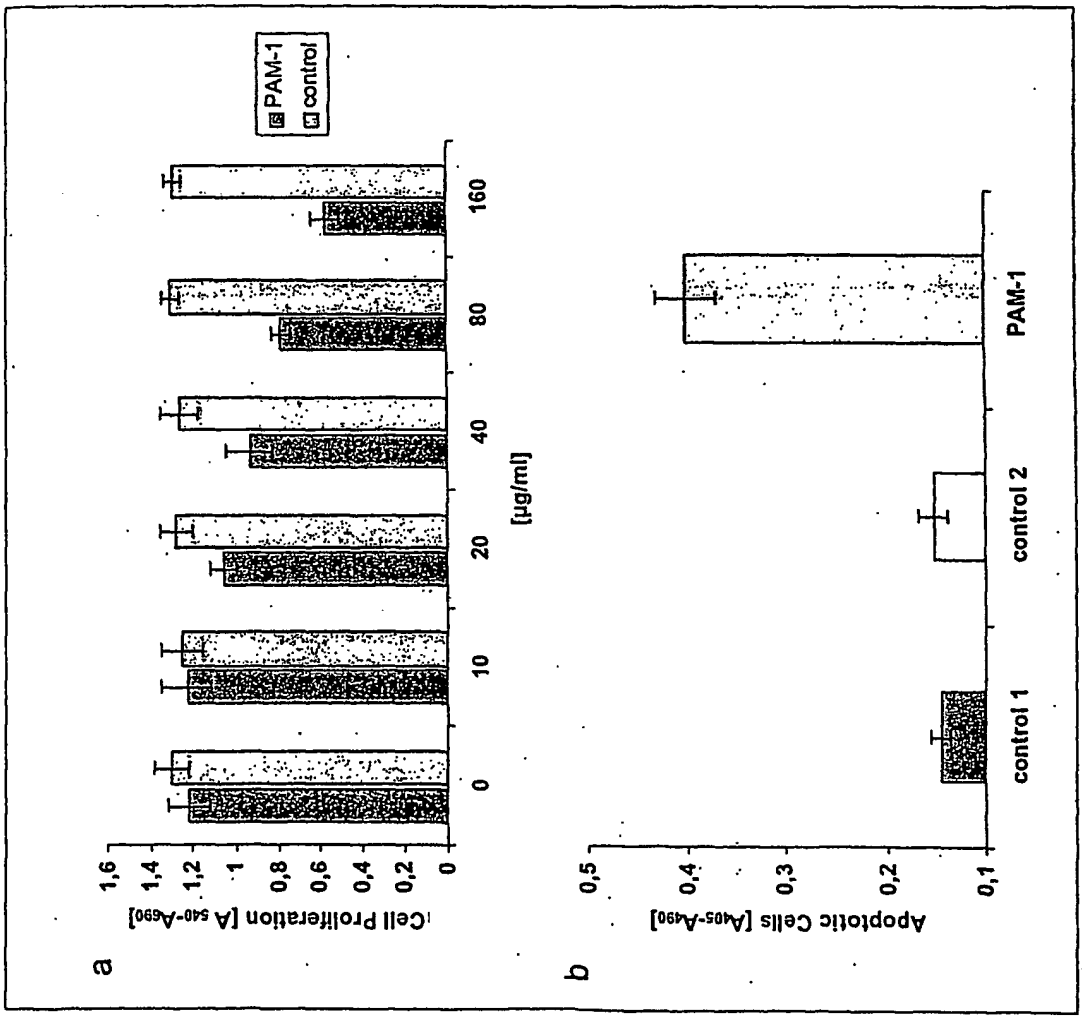
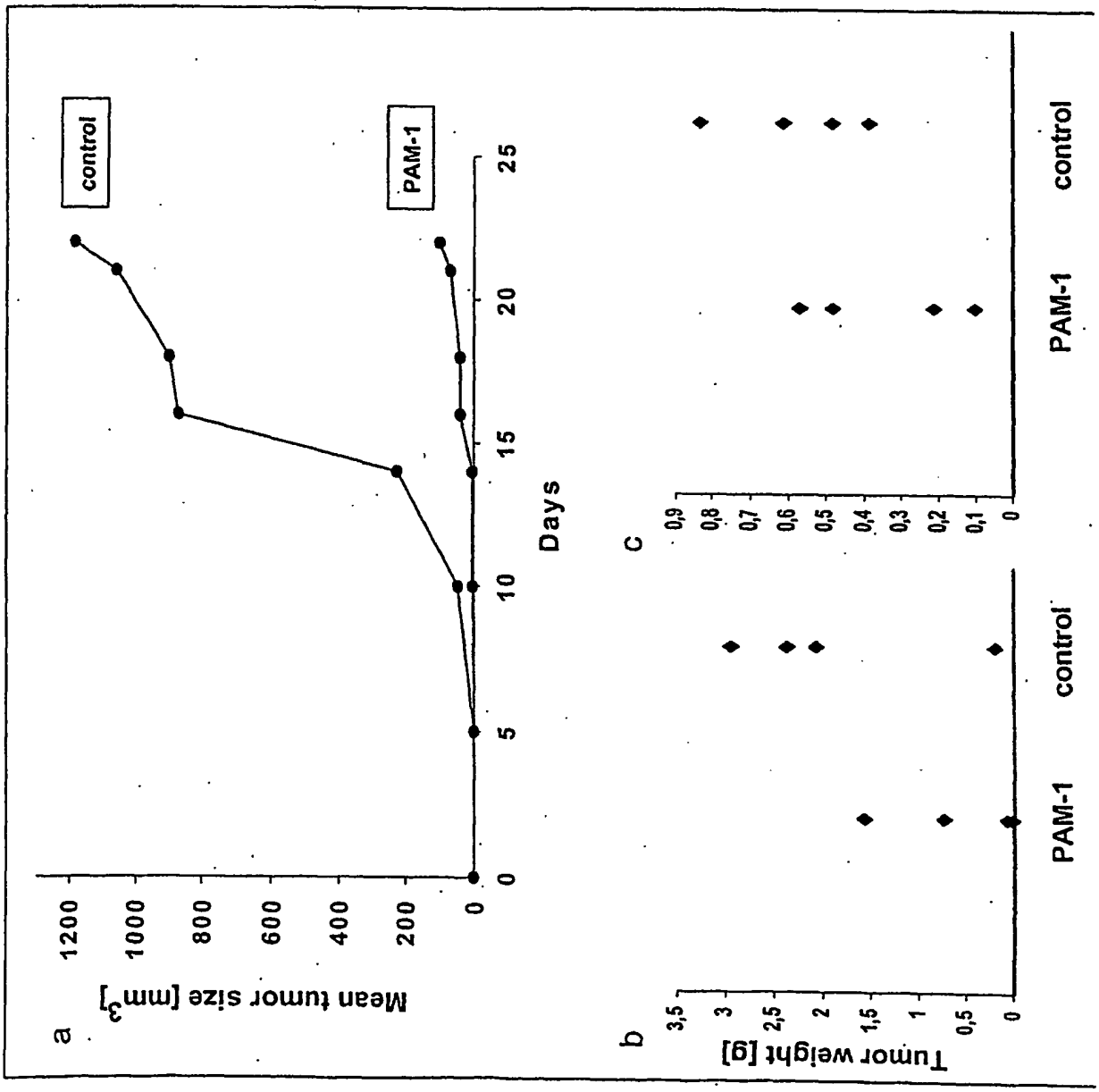

a
Cell Proliferation [A 540-A690]
1,6
1,4
1,2
1
0,8
0,6
0,4
0,2
0
0
10
20
40
80
160
[µg/ml]
PAM-1
control
b
Apoptotic Cells [A405-A490]
0,5
0,4
0,3
0,2
0,1
control 1
control 2
PAM-1

Fig. 14


a
Mean tumor size [mm3]
1200
1000
800
600
400
200
0
control
PAM-1
0
5
10
15
20
25
Days
b
Tumor weight [g]
3,5
3
2,5
2
1,5
1
0,5
0
PAM-1
control
c
0,9
0,8
0,7
0,6
0,5
0,4
0,3
0,2
0,1
0
PAM-1
control

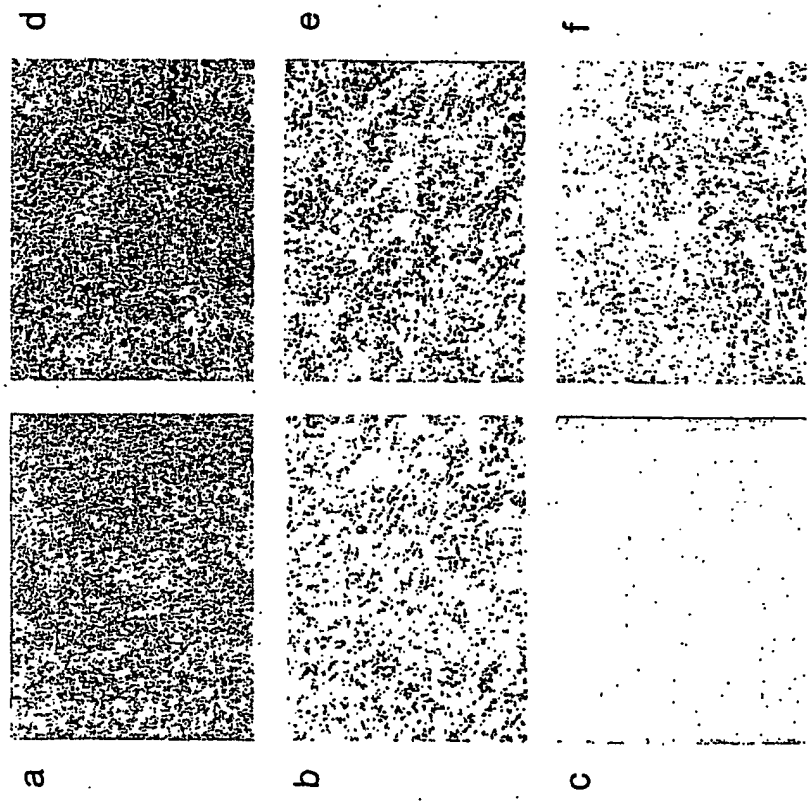


Fig. 15

```
23132     1 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
CFR-1     1 MAACGRVRRM FRLSAALHLL LLFAAGGRNS PARASHSQGQ GPGANFVSFV GQAGGGGPAG QQLPQLPQSS QLQQQQQQQQ QQQQPQPPQP PFPAGGPP-R
MG160     1 MAACGRVRRM FRLSAALHLL LLFAAGAEKL PGHGVHSQGQ GPGANFVSFV GQAGGGGPAG QQLPQLLQSS QLQQQQQQQQ QQQQLQPPQP PFPAGGPPAR

23132   101 ---------- ---------- ---------- ---------- --DVREPENE ISSDCNHLLW NYKLNLTTDP KFESVAREVC KSTITEIREC ADEPVGKGYM
CFR-1   101 RGGAGAGGGW KLAEEESCRE DVTRVCPKHT WSNNLAVLEC LQDVREPENE ISSDCNHLLW NYKLNLTTDP KFESVAREVC KSTITEIKEC ADEPVGKGYM
MG160   101 RGGAGAGGGW KLAEEESCRE DVTRVCPKHT WSNNLAVLEC LQDVREPENE ISSDCNHLLW NYKLNLTTDP KFESVAREVC KSTITEIKEC ADEPVGKGYM

23132   201 VSCLVDHRGN ITEYQCHQYI TKMTAIIFSD YRLICGFMDD CKNDINILKC GSIRLGEKDA HSQGEVVSCL EKGLVKEAEE REPKIQVSEL CKKAILRVAE
CFR-1   201 VSCLVDHRGN ITEYQCHQYI TKMTAIIFSD YRLICGFMDD CKNDINILKC GSIRLGEKDA HSQGEVVSCL EKGLVKEAEE REPKIQVSEL CKKAILRVAE
MG160   201 VSCLVDHRGN ITEYQCHQYI TKMTAIIFSD YRLICGFMDD CKNDINILKC GSIRLGEKDA HSQGEVVSCL EKGLVKEAEE REPKIQVSEL CKKAILRVAE

23132   301 LSSDDFHLDR HLYFACRDDR ERFCENTQAG EGRVYKCLFN HKFEESMSEK CREALTTRQK LIAQDYKVSY SLAKSCKSDL KKYRCNVENL PRSREARLSY
CFR-1   301 LSSDDFHLDR HLYFACRDDR ERFCENTQAG EGRVYKCLFN HKFEESMSEK CREALTTRQK LIAQDYKVSY SLAKSCKSDL KKYRCNVENL PRSREARLSY
MG160   301 LSSDDFHLDR HLYFACRDDR ERFCENTQAG EGRVYKCLFN HKFEESMSEK CREALTTRQK LIAQDYKVSY SLAKSCKSDL KKYRCNVENL PRSREARLSY

23132   401 LLMCLESAVH RGRQVSSECQ GEMLDYRRML MEDFSLSPEI ILSCRGEIEH HCSGLHRKGR TLHCLMKVVR GEKGNLGMNC QQALQTLIQE TDPGADYRID
CFR-1   401 LLMCLESAVH RGRQVSSECQ GEMLDYRRML MEDFSLSPEI ILSCRGEIEH HCSGLHRKGR TLHCLMKVVR GEKGNLGMNC QQALQTLIQE TDPGADYRID
MG160   401 LLMCLESAVH RGRQVSSECQ GEMLDYRRML MEDFSLSPEI ILSCRGEIEH HCSGLHRKGR TLHCLMKVVR GEKGNLGMNC QQALQTLIQE TDPGADYRID

23132   501 RALNEACESV IQTACKHIRS GDPMILSCLM EHLYTEKMVE DCEHRLLELQ YFISRDWKLD PVLYRKCQGD ASRLCHTHGW NETSEFMPQG AVFSCLYRHA
CFR-1   501 RALNEACESV IQTACKHIRS GDPMILSCLM EHLYTEKMVE DCEHRLLELQ YFISRDWKLD PVLYRKCQGD ASRLCHTHGW NETSEFMPQG AVFSCLYRHA
MG160   501 RALNEACESV IQTACKHIRS GDPMILSCLM EHLYTEKMVE DCEHRLLELQ YFISRDWKLD PVLYRKCQGD ASRLCHTHGW NETSEFMPQG AVFSCLYRHA

23132   601 YRTEEQGRRL SRECRAEVQR ILHQRAMDVK LDPALQDKCL IDLGKWCSEK TETGQELECL QDHLDDLVVE CRDIVGNLTE LESEDIQIEA LLMRACEPII
CFR-1   601 YRTEEQGRRL SRECRAEVQR ILHQRAMDVK LDPALQDKCL IDLGKWCSEK TETGQELECL QDHLDDLVVE CRDIVGNLTE LESEDIQIEA LLMRACEPII
MG160   601 YRTEEQGRRL LDPALQDKCL IDLGKWCSEK TETGQELECL SRECRAEVQR ILHQRAMDVK QDHLDDLVVE CRDIVGNLTE LESEDIQIEA LLMRACEPII

23132   701 QNFCHDVADN QIDSGDLMEC LIQNKHQKDM NEKCAIGVTH FQLVQMKDFR FSYKFKMACK EDVLKLCPNI KKKVDVVICL STTVRNDTLQ EAKEHRVSLK
CFR-1   701 QTFCHDVADN QIDSGDLMEC LIQNKHQKDM NEKCAIGVTH FQLVQMKDFR FSYKFKMACK EDVLKLCPNI KKKVDVVICL STTVRNDTLQ EAKEHRVSLK
MG160   701 QNFCHDVADN QIDSGDLMEC LIQNKHQKDM NEKCAIGVTH FQLVQMKDFR FSYKFKMACK EDVLKLCPNI KKKVDVVICL STTVRNDTLQ EAKEHRVSLK

23132   801 CRRQLRVEEL EMTEDIRLEP DLYEACKSDI KNFCSAVQYG NAQIIECLKE NKKQLSTRCH QKVFKLQETE MMDPELDYTL MRVCKQMIKK FCPEADSKTM
CFR-1   801 CRRQLRVEEL EMTEDIRLEP DLYEACKSDI KNFCSAVQYG NAQIIECLKE NKKQLSTRCH QKVFKLQETE MMDPELDYTL MRVCKQMIKR FCPEADSKTM
MG160   801 CRRQLRVEEL EMTEDIRLEP DLYEACKSDI KNFCSAVQYG NAQIIECLKE NKKQLSTRCH QKVFKLQETE MMDPELDYTL MRVCKQMIKR FCPEADSKTM

23132   901 LQCLKQNKNS ELMDPKCKQM ITKRQITQNT DYRLNPMLRK ACKADIPKFC HGILTKAKDD SELEGQVISC LKLRYADQRL SSDCEDQIRI IIQESALDYR
CFR-1   901 LQCLKQNKNS ELMDPKCKQM ITKRQITQNT DYRLNPMLRK ACKADIPKFC HGILTKAKDD SELEGQVISC LKLRYADQRL SSDCEDQIRI IIQESALDYR
MG160   901 LQCLKQNKNS ELMDPKCKQM ITKRQITQNT DYRLNPMLRK ACKADIPKFC HGILTKAKDD SELEGQVISC LKLRYADQRL SSDCEDQIRI IIQESALDYR

23132  1001 LDPQLQLHCS DEISSLCAEE AAAQEQTGQV EECLKVNLLK IKTELCKKEV LNMLKESKAD IFVDPVLHTA CALDIKHHCA AITPGRGRQM SCLMEALEDK
CFR-1  1001 LDPQLQLHCS DEISSLCAEE AAAQEQTGQV EECLKVNLLK IKTELCKKEV LNMLKESKAD IFVDPVLHTA CALDIKHHCA AITPGRGRQM SCLMEALEDK
MG160  1001 LDPQLQLHCS DEISSLCAEE AAAQEQTGQV EECLKVNLLK IKTELCKKEV LNMLKESKAD IFVDPVLHTA CALDIKHHCA AITPGRGRQM SCLMEALEDK

23132  1101 RVRLQPECKK RLNDRIEMWS YAAKVAPADG FSDLAMQVMT SPSKNYILSV ISGSICILFL IGLMCGRITK RVTRELKDR*
CFR-1  1101 RVRLQPECKK RLNDRIEMWS YAAKVAPADG FSDLAMQVMT SPSKNYILSV ISGSICILFL IGLMCGRITK RVTRELKDR*
MG160  1101 RVRLQPECKK RLNDRIEMWS YAAKVAPADG FSDLAMQVMT SPSKNYILSV ISGSICILFL IGLMCGRITK RVTRELKDR*
```

Human antibody PAM-1 (clone 103/51)

Heavy chain sequence

CDR I

```
AGG TCC CTG AGA CTC TCC TGT GCA GCC TCT GGA TTC ACC TTC AGT AGC TAT GGC    54
Arg Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr Gly
                5                  10                  15

ATG CAC TGG GTC CGC CAG GCT CCA GGC AAG GGG CTG GAG TGG GTG GCA GTT ATA   108
Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ala Val Ile
    20                  25                  30                  35

      CDR II

TCA TAT GAT GGA AGT AAT AAA TAC TAT GCA GAC TCC GTG AAG GGC CGA TTC ACC   162
Ser Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val Lys Gly Arg Phe Thr
            40                  45                  50

ATC TCC AGA GAC AAT TCC AAG AAC ACG CTG TAT CTG CAA ATG AAC AGC CTG AGA   216
Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg
55                  60                  65                  70
                                                CDR III

GCT GAG GAC ACG GCT GTG TAT TAC TGT GCG AGG TCG ACT ACG AGG TCT TAT CCT   270
Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg Ser Thr Thr Arg Ser Tyr Pro
        75                  80                  85                  90

                    CDR III

CTA TAC GGT ATG GAC GTT TGG GGC CAA GGG AAC CCT GTC ACC       312
Leu Tyr Gly Met Asp Val Trp Gly Gln Gly Asn Pro Val Thr
                95                  100
```

Fig. 17

Human antibody PAM-1 (clone 103/51)

Light chain sequence

```
GTG ACC TCC TAT GTG CTG ACT CAG CCA CCC TCG GTG TCA GTG GCC CCA GGA CAG    54
Val Thr Ser Tyr Val Leu Thr Gln Pro Pro Ser Val Ser Val Ala Pro Gly Gln
                  5                  10                  15
                                        CDR I
                                    ___________________
ACG GCC AGT ATT ACC TGT GGG GGA AAT AAC ATT GGA AGT AAA AGT GTG CAC TGG   108
Thr Ala Ser Ile Thr Cys Gly Gly Asn Asn Ile Gly Ser Lys Ser Val His Trp
      20                  25                  30                  35
                                                          CDR II
                                                        ___________
TAC CAT CAG AAG CCA GGC CAG GCC CCT GTG CTG GTC GTC TAT GAT GAT AGC GAC   162
Tyr His Gln Lys Pro Gly Gln Ala Pro Val Leu Val Val Tyr Asp Asp Ser Asp
             40                  45                  50
CGG CCC TCA GGG ATC CCT GAG CGA TTC TCT GGC TCC AAC TCT GGG AAC ACG GCC   216
Arg Pro Ser Gly Ile Pro Glu Arg Phe Ser Gly Ser Asn Ser Gly Asn Thr Ala
 55                  60                  65                  70
ACC CTG ACC ATC ACC AGG GTC GAA GCC GGG GAT GAG GCC GAC TAT TAC TGT CAG   270
Thr Leu Thr Ile Thr Arg Val Glu Ala Gly Asp Glu Ala Asp Tyr Tyr Cys Gln
         75                  80                  85                  90
                  CDR III
_____________________________________
GTG TGG GAT AGT AGT AGT GAT CTC AAT TGG GTG TTC GGC GGA AGG ACC CAA GCT   324
Val Trp Asp Ser Ser Ser Asp Leu Asn Trp Val Phe Gly Gly Arg Thr Gln Ala
                  95                  100                 105

GAC CGT CCT ACG TCA GCC CAA GGC TGC CCC TCC GGT CAC TCT GTT CCC CGC CCC   378
Asp Arg Pro Thr Ser Ala Gln Gly Cys Pro Ser Gly His Ser Val Pro Arg Pro
     110                 115                 120                 125

CCT CTG AAG AGC TTC AAG CTT  399
Pro Leu Lys Ser Phe Lys Leu
            130
```

Fig. 18

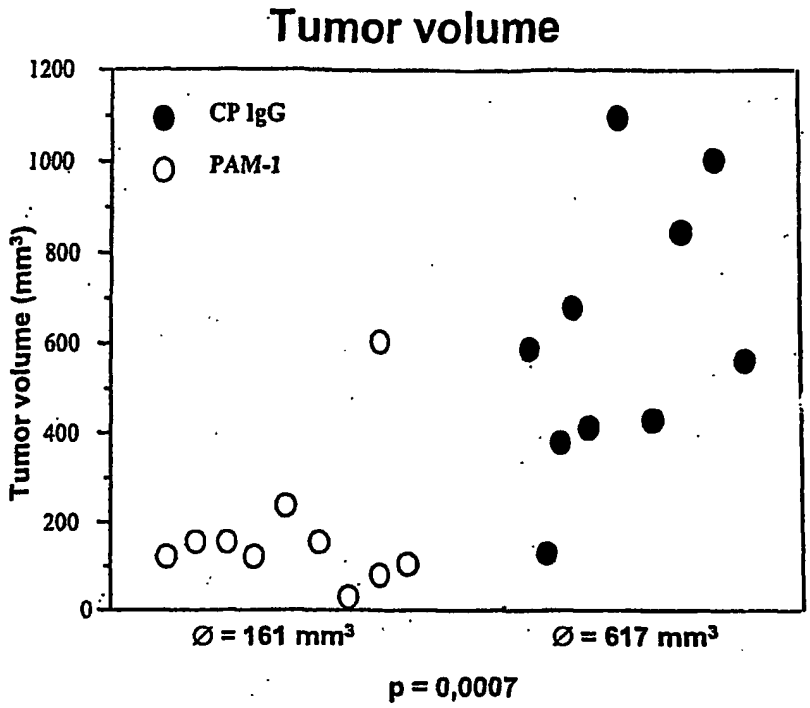


Fig. 19(A)

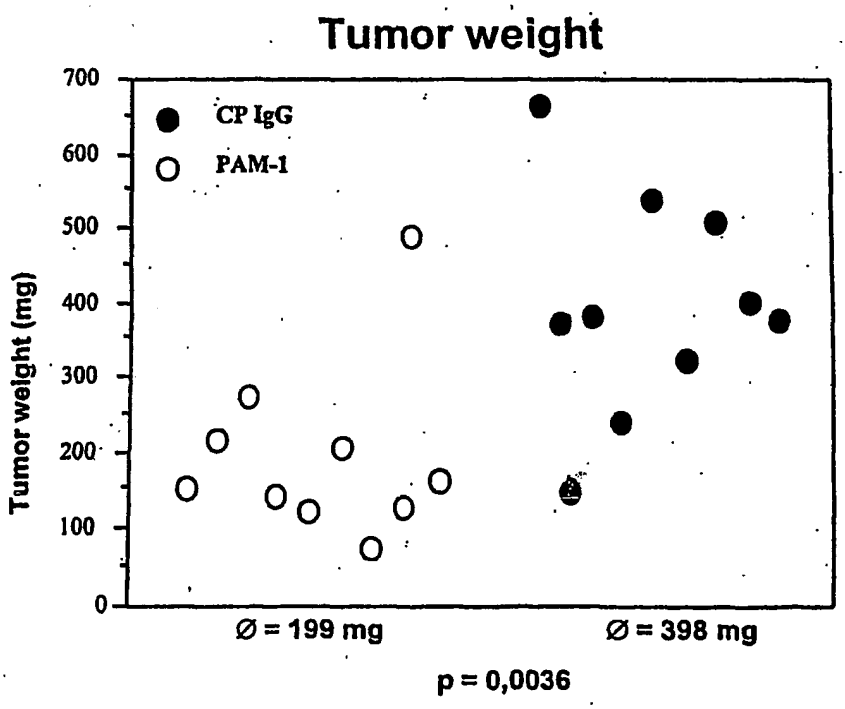


Fig. 19(B)

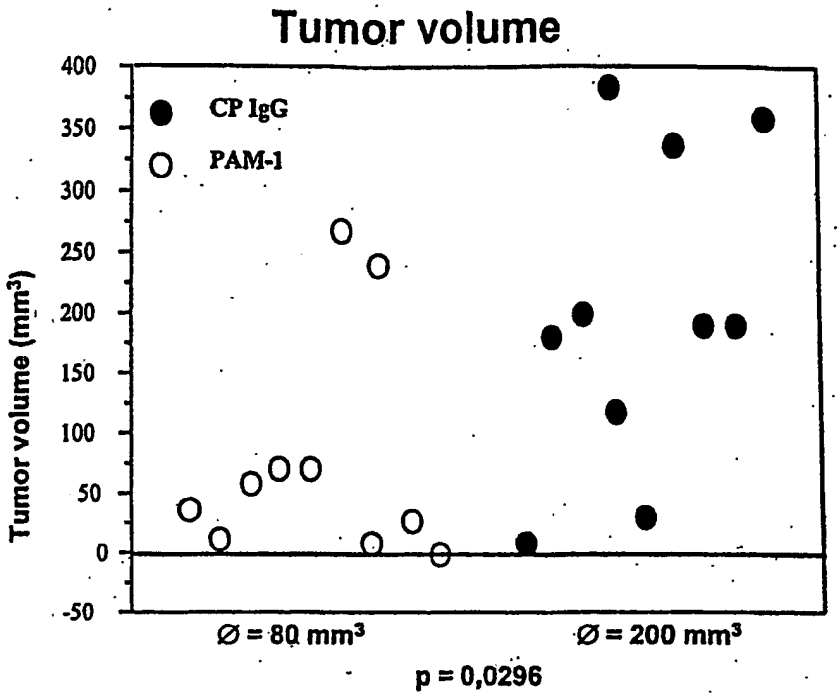


Fig. 20(A)

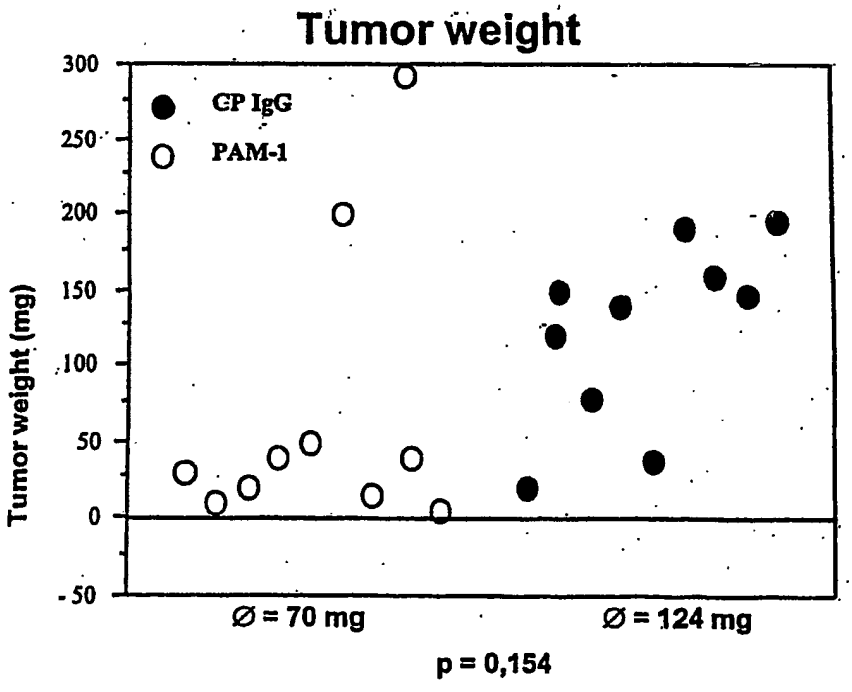


Fig. 20(B)

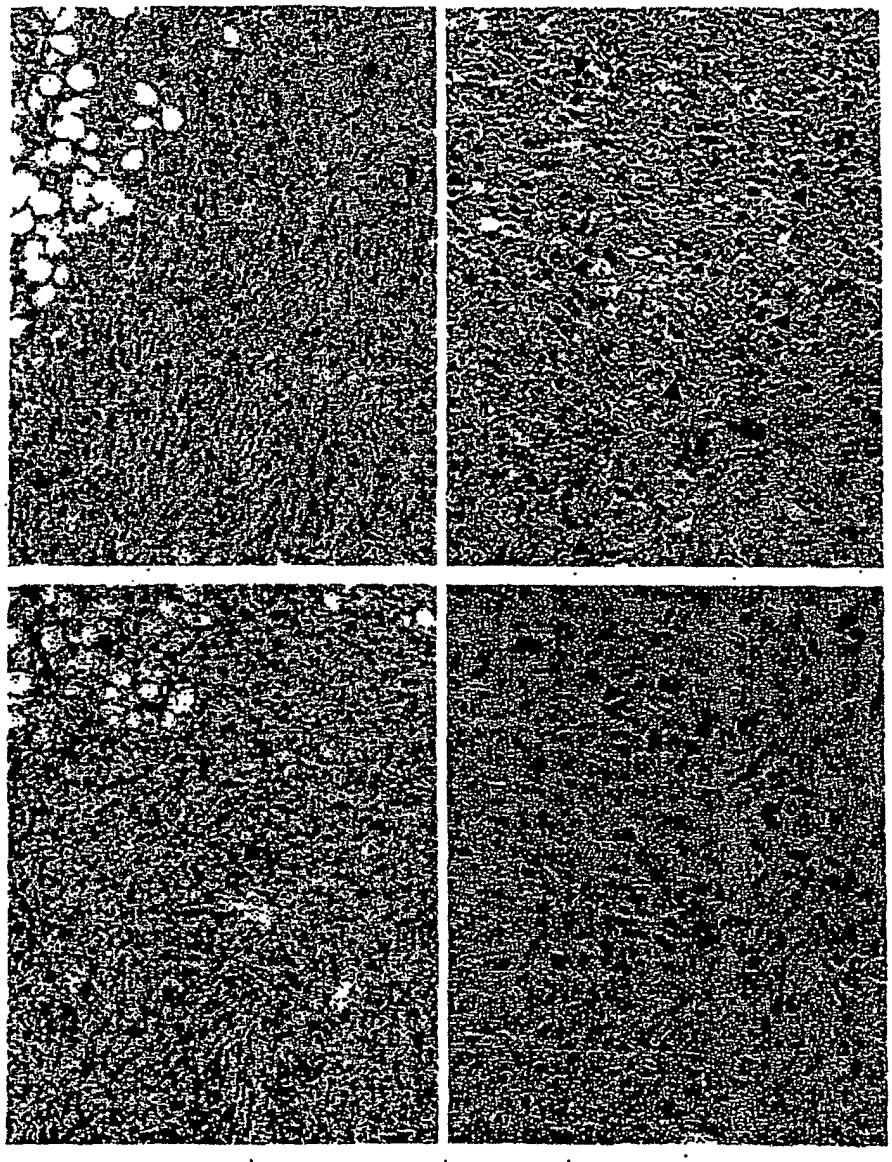


Fig. 21(A) Fig. 21(B) Fig. 21(C) Fig. 21(D)

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<120> Neoplasm-Specific Polypeptides and Their Uses

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<150> 10/764,730
<151> 2004-01-26

<160> 30

<170> FastSEQ for Windows Version 4.0

<210> 1
<211> 288
<212> DNA
<213> Mus musculus

<220>
<221> CDS
<222> (1)...(288)

<400> 1

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tcc tgc aag gct tct ggc tac acc ttc act gac tac tat ata aac tgg   48
Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asp Tyr Tyr Ile Asn Trp
 1               5                  10                  15

gtg aag cag agg act gga cag ggc ctt gag tgg att gga gag att tat   96
Val Lys Gln Arg Thr Gly Gln Gly Leu Glu Trp Ile Gly Glu Ile Tyr
            20                  25                  30

cct gga agt ggt aat act tac tac aat gag aag ttc aag ggc aag gcc   144
Pro Gly Ser Gly Asn Thr Tyr Tyr Asn Glu Lys Phe Lys Gly Lys Ala
        35                  40                  45

aca ctg act gca gac aaa tcc tcc agc aca gcc tac atg cag ctc agc   192
Thr Leu Thr Ala Asp Lys Ser Ser Ser Thr Ala Tyr Met Gln Leu Ser
    50                  55                  60

agc ctg aca tct gag gac tct gca gtc tat ttc tgt gca aga tcg gga   240
Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Phe Cys Ala Arg Ser Gly
65                  70                  75                  80

tta cga ccc tat gct atg gac tac tgg ggt caa gga acc tca gtc acc   288
Leu Arg Pro Tyr Ala Met Asp Tyr Trp Gly Gln Gly Thr Ser Val Thr
                85                  90                  95
```

<210> 2
<211> 96
<212> PRT
<213> Mus musculus

<400> 2

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Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asp Tyr Tyr Ile Asn Trp
 1               5                  10                  15
Val Lys Gln Arg Thr Gly Gln Gly Leu Glu Trp Ile Gly Glu Ile Tyr
            20                  25                  30
Pro Gly Ser Gly Asn Thr Tyr Tyr Asn Glu Lys Phe Lys Gly Lys Ala
        35                  40                  45
Thr Leu Thr Ala Asp Lys Ser Ser Ser Thr Ala Tyr Met Gln Leu Ser
    50                  55                  60
Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Phe Cys Ala Arg Ser Gly
65                  70                  75                  80
Leu Arg Pro Tyr Ala Met Asp Tyr Trp Gly Gln Gly Thr Ser Val Thr
                85                  90                  95
```

<210> 3
<211> 315
<212> DNA
<213> Mus musculus

<220>
<221> CDS
<222> (1)...(315)

<400> 3

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cca ctc tcc ctg cct gtc agt ctt gga gat caa gcc tcc atc tct tgc   48
Pro Leu Ser Leu Pro Val Ser Leu Gly Asp Gln Ala Ser Ile Ser Cys
 1               5                  10                  15

aga tct agt cag agc att gta cat agt aat gga aac acc tat tta gaa   96
Arg Ser Ser Gln Ser Ile Val His Ser Asn Gly Asn Thr Tyr Leu Glu
            20                  25                  30

tgg tac ctg cag aaa cca ggc cag tct cca aag ctc ctg atc tac aaa   144
Trp Tyr Leu Gln Lys Pro Gly Gln Ser Pro Lys Leu Leu Ile Tyr Lys
        35                  40                  45

gtt tcc aac cga ttt tct ggg gtc cca gac agg ttc agt ggc agt gga   192
Val Ser Asn Arg Phe Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly
    50                  55                  60

tca ggg aca gat ttc aca ctc aag atc agc aga gtg gag gct gag gat   240
Ser Gly Thr Asp Phe Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp
65                  70                  75                  80

ctg gga gtt tat tac tgc ttt caa ggt tca cat gtt ccg tac acg ttc   288
Leu Gly Val Tyr Tyr Cys Phe Gln Gly Ser His Val Pro Tyr Thr Phe
                85                  90                  95

gga ggg ggg acc aag ctg gaa ata aaa                              315
Gly Gly Gly Thr Lys Leu Glu Ile Lys
            100                 105
```

<210> 4
<211> 105

<212> PRT
<213> Mus musculus

<400> 4

```
Pro Leu Ser Leu Pro Val Ser Leu Gly Asp Gln Ala Ser Ile Ser Cys
 1               5                  10                  15
Arg Ser Ser Gln Ser Ile Val His Ser Asn Gly Asn Thr Tyr Leu Glu
            20                  25                  30
Trp Tyr Leu Gln Lys Pro Gly Gln Ser Pro Lys Leu Leu Ile Tyr Lys
        35                  40                  45
Val Ser Asn Arg Phe Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly
    50                  55                  60
Ser Gly Thr Asp Phe Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp
65                  70                  75                  80
Leu Gly Val Tyr Tyr Cys Phe Gln Gly Ser His Val Pro Tyr Thr Phe
                85                  90                  95
Gly Gly Gly Thr Lys Leu Glu Ile Lys
            100                 105
```

<210> 5
<211> 3114
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)...(3114)

<400> 5

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gat gtg agg gag cct gaa aat gaa att tct tca gac tgc aat cat ttg   48
Asp Val Arg Glu Pro Glu Asn Glu Ile Ser Ser Asp Cys Asn His Leu
 1               5                  10                  15

ttg tgg aat tat aag ctg aac cta act aca gat ccc aaa ttt gaa tct   96
Leu Trp Asn Tyr Lys Leu Asn Leu Thr Thr Asp Pro Lys Phe Glu Ser
            20                  25                  30

gtg gcc aga gag gtt tgc aaa tct act ata aca gag att gaa gaa tgt   144
Val Ala Arg Glu Val Cys Lys Ser Thr Ile Thr Glu Ile Glu Glu Cys
        35                  40                  45

gct gat gaa ccg gtt gga aaa ggt tac atg gtt tcc tgc ttg gtg gat   192
Ala Asp Glu Pro Val Gly Lys Gly Tyr Met Val Ser Cys Leu Val Asp
    50                  55                  60

cac cga ggc aac atc act gag tat cag tgt cac cag tac att acc aag   240
His Arg Gly Asn Ile Thr Glu Tyr Gln Cys His Gln Tyr Ile Thr Lys
65                  70                  75                  80

atg acg gcc atc att ttt agt gat tac cgt tta atc tgt ggc ttc atg   288
Met Thr Ala Ile Ile Phe Ser Asp Tyr Arg Leu Ile Cys Gly Phe Met
                85                  90                  95

gat gac tgc aaa aat gac atc aac att ctg aaa tgt ggc agt att cgg   336
Asp Asp Cys Lys Asn Asp Ile Asn Ile Leu Lys Cys Gly Ser Ile Arg
            100                 105                 110
```

```
ctt gga gaa aag gat gca cat tca caa ggt gag gtg gta tca tgc ttg   384
Leu Gly Glu Lys Asp Ala His Ser Gln Gly Glu Val Val Ser Cys Leu
        115                 120                 125

gag aaa ggc ctg gtg aaa gaa gca gaa gaa aga gaa ccc aag att caa   432
Glu Lys Gly Leu Val Lys Glu Ala Glu Glu Arg Glu Pro Lys Ile Gln
    130                 135                 140

gtt tct gaa ctc tgc aag aaa gcc att ctc cgg gtg gct gag ctg tca   480
Val Ser Glu Leu Cys Lys Lys Ala Ile Leu Arg Val Ala Glu Leu Ser
145                 150                 155                 160

tcg gat gac ttt cac tta gac cgg cat tta tat ttt gct tgc cga gat   528
Ser Asp Asp Phe His Leu Asp Arg His Leu Tyr Phe Ala Cys Arg Asp
                165                 170                 175

gat cgg gag cgt ttt tgt gaa aat aca caa gct ggt gag ggc aga gtg   576
Asp Arg Glu Arg Phe Cys Glu Asn Thr Gln Ala Gly Glu Gly Arg Val
            180                 185                 190

tat aag tgc ctc ttt aac cat aaa ttt gaa gaa tcc atg agt gaa aag   624
Tyr Lys Cys Leu Phe Asn His Lys Phe Glu Glu Ser Met Ser Glu Lys
        195                 200                 205

tgt cga gaa gca ctt aca acc cgc caa aag ctg att gcc cag gat tat   672
Cys Arg Glu Ala Leu Thr Thr Arg Gln Lys Leu Ile Ala Gln Asp Tyr
    210                 215                 220

aaa gtc agt tat tca ttg gcc aaa tcc tgt aaa agt gac ttg aag aaa   720
Lys Val Ser Tyr Ser Leu Ala Lys Ser Cys Lys Ser Asp Leu Lys Lys
225                 230                 235                 240

tac cgg tgc aat gtg gaa aac ctt ccg cga tcg cgt gaa gcc agg ctc   768
Tyr Arg Cys Asn Val Glu Asn Leu Pro Arg Ser Arg Glu Ala Arg Leu
                245                 250                 255

tcc tac ttg tta atg tgc ctg gag tca gct gta cac aga ggg cga caa   816
Ser Tyr Leu Leu Met Cys Leu Glu Ser Ala Val His Arg Gly Arg Gln
            260                 265                 270

gtc agc agt gag tgc cag ggg gag atg ctg gat tac cga cgc atg ttg   864
Val Ser Ser Glu Cys Gln Gly Glu Met Leu Asp Tyr Arg Arg Met Leu
        275                 280                 285

atg gaa gac ttt tct ctg agc cct gag atc atc cta agc tgt cgg ggg   912
Met Glu Asp Phe Ser Leu Ser Pro Glu Ile Ile Leu Ser Cys Arg Gly
    290                 295                 300

gag att gaa cac cat tgt tcc gga tta cat cga aaa ggg cgg acc cta   960
Glu Ile Glu His His Cys Ser Gly Leu His Arg Lys Gly Arg Thr Leu
305                 310                 315                 320

cac tgt ctg atg aaa gta gtt cga ggg gag aag ggg aac ctt gga atg   1008
His Cys Leu Met Lys Val Val Arg Gly Glu Lys Gly Asn Leu Gly Met
                325                 330                 335
```

```
aac tgc cag cag gcg ctt caa aca ctg att cag gag act gac cct ggt   1056
Asn Cys Gln Gln Ala Leu Gln Thr Leu Ile Gln Glu Thr Asp Pro Gly
            340                 345                 350

gca gat tac cgc att gat cga gct ttg aat gaa gct tgt gaa tct gta   1104
Ala Asp Tyr Arg Ile Asp Arg Ala Leu Asn Glu Ala Cys Glu Ser Val
        355                 360                 365

atc cag aca gcc tgc aaa cat ata aga tct gga gac cca atg atc ttg   1152
Ile Gln Thr Ala Cys Lys His Ile Arg Ser Gly Asp Pro Met Ile Leu
    370                 375                 380

tcg tgc ctg atg gaa cat tta tac aca gag aag atg gta gaa gac tgt   1200
Ser Cys Leu Met Glu His Leu Tyr Thr Glu Lys Met Val Glu Asp Cys
385                 390                 395                 400

gaa cac cgt ctc tta gag ctg cag tat ttc atc tcc cgg gat tgg aag   1248
Glu His Arg Leu Leu Glu Leu Gln Tyr Phe Ile Ser Arg Asp Trp Lys
                405                 410                 415

ctg gac cct gtc ctg tac cgc aag tgc cag gga gac gct tct cgt ctt   1296
Leu Asp Pro Val Leu Tyr Arg Lys Cys Gln Gly Asp Ala Ser Arg Leu
            420                 425                 430

tgc cac acc cac ggt tgg aat gag acc agc gaa ttt atg cct cag gga   1344
Cys His Thr His Gly Trp Asn Glu Thr Ser Glu Phe Met Pro Gln Gly
        435                 440                 445

gct gtg ttc tct tgt tta tac aga cac gcc tac cgc act gag gaa cag   1392
Ala Val Phe Ser Cys Leu Tyr Arg His Ala Tyr Arg Thr Glu Glu Gln
    450                 455                 460

gga agg agg ctc tca cgg gag tgc cga gct gaa gtc caa agg atc cta   1440
Gly Arg Arg Leu Ser Arg Glu Cys Arg Ala Glu Val Gln Arg Ile Leu
465                 470                 475                 480

cac cag cgt gcc atg gat gtc aag ctg gat cct gcc ctc cag gat aag   1488
His Gln Arg Ala Met Asp Val Lys Leu Asp Pro Ala Leu Gln Asp Lys
                485                 490                 495

tgc ctg att gat ctg gga aaa tgg tgc agt gag aaa aca gag act gga   1536
Cys Leu Ile Asp Leu Gly Lys Trp Cys Ser Glu Lys Thr Glu Thr Gly
            500                 505                 510

cag aag ctg gag tgc ctt cag gac cat ctg gat gac tta gtg gtg gag   1584
Gln Lys Leu Glu Cys Leu Gln Asp His Leu Asp Asp Leu Val Val Glu
        515                 520                 525

tgt aga gat ata gtt ggc aac ctc act gag tta gaa tca gag gat att   1632
Cys Arg Asp Ile Val Gly Asn Leu Thr Glu Leu Glu Ser Glu Asp Ile
    530                 535                 540

caa ata gaa gcc ttg ctg atg aga gcc tgt gag ccc ata att cag aac   1680
Gln Ile Glu Ala Leu Leu Met Arg Ala Cys Glu Pro Ile Ile Gln Asn
545                 550                 555                 560

ttc tgc cac gat gtg gca gat aac cag ata gac tcc ggg gac ctg atg   1728
```

```
Phe Cys His Asp Val Ala Asp Asn Gln Ile Asp Ser Gly Asp Leu Met
                565                 570                 575

gag tgt ctg ata cag aac aaa cac cag aag gac atg aac gag aag tgt   1776
Glu Cys Leu Ile Gln Asn Lys His Gln Lys Asp Met Asn Glu Lys Cys
            580                 585                 590

gcc atc gga gtt acc cac ttc cag ctg gtg cag atg aag gat ttt cgg   1824
Ala Ile Gly Val Thr His Phe Gln Leu Val Gln Met Lys Asp Phe Arg
        595                 600                 605

ttt tct tac aag ttt aaa atg gcc tgc aag gag gac gtg ttg aag ctt   1872
Phe Ser Tyr Lys Phe Lys Met Ala Cys Lys Glu Asp Val Leu Lys Leu
    610                 615                 620

tgc cca aac ata aaa aag aag gtg gac gtg gtg atc tgc ctg agc acg   1920
Cys Pro Asn Ile Lys Lys Lys Val Asp Val Val Ile Cys Leu Ser Thr
625                 630                 635                 640

acc gtg cgc aat gac act ctg cag gaa gcc aag gag cac agg gtg tcc   1968
Thr Val Arg Asn Asp Thr Leu Gln Glu Ala Lys Glu His Arg Val Ser
                645                 650                 655

ctg aag tgc cgc agg cag ctc cgt gtg gag gag ctg gag atg acg gag   2016
Leu Lys Cys Arg Arg Gln Leu Arg Val Glu Glu Leu Glu Met Thr Glu
            660                 665                 670

gac atc cgc ttg gag cca gat cta tac gaa gcc tgc aag agt gac atc   2064
Asp Ile Arg Leu Glu Pro Asp Leu Tyr Glu Ala Cys Lys Ser Asp Ile
        675                 680                 685

aaa aac ttc tgt tcc gct gtg caa tat ggc aac gct cag att atc gaa   2112
Lys Asn Phe Cys Ser Ala Val Gln Tyr Gly Asn Ala Gln Ile Ile Glu
    690                 695                 700

tgt ctg aaa gaa aac aag aag cag cta agc acc cgc tgc cac caa aaa   2160
Cys Leu Lys Glu Asn Lys Lys Gln Leu Ser Thr Arg Cys His Gln Lys
705                 710                 715                 720

gta ttt aag ctg cag gag aca gag atg atg gac cca gag cta gac tac   2208
Val Phe Lys Leu Gln Glu Thr Glu Met Met Asp Pro Glu Leu Asp Tyr
                725                 730                 735

acc ctc atg agg gtc tgc aag cag atg ata aag aag ttc tgt ccg gaa   2256
Thr Leu Met Arg Val Cys Lys Gln Met Ile Lys Lys Phe Cys Pro Glu
            740                 745                 750

gca gat tct aaa acc atg ttg cag tgc ttg aag caa aat aaa aac agt   2304
Ala Asp Ser Lys Thr Met Leu Gln Cys Leu Lys Gln Asn Lys Asn Ser
        755                 760                 765

gaa ttg atg gat ccc aaa tgc aaa cag atg ata acc aag cgc cag atc   2352
Glu Leu Met Asp Pro Lys Cys Lys Gln Met Ile Thr Lys Arg Gln Ile
    770                 775                 780

acc cag aac aca gat tac cgc tta aac ccc atg tta aga aaa gcc tgt   2400
Thr Gln Asn Thr Asp Tyr Arg Leu Asn Pro Met Leu Arg Lys Ala Cys
```

```
785                 790                 795                 800

aaa gct gac att cct aaa ttc tgt cac ggt atc ctg act aag gcc aag   2448
Lys Ala Asp Ile Pro Lys Phe Cys His Gly Ile Leu Thr Lys Ala Lys
                805                 810                 815

gat gat tca gaa tta gaa gga caa gtc atc tct tgc ctg aag ctg aga   2496
Asp Asp Ser Glu Leu Glu Gly Gln Val Ile Ser Cys Leu Lys Leu Arg
            820                 825                 830

tat gct gac cag cgc ctg tct tca gac tgt gaa gac cag atc cga atc   2544
Tyr Ala Asp Gln Arg Leu Ser Ser Asp Cys Glu Asp Gln Ile Arg Ile
        835                 840                 845

att atc cag gag tcc gcc ctg gac tac cgc ctg gat cct cag ctc cag   2592
Ile Ile Gln Glu Ser Ala Leu Asp Tyr Arg Leu Asp Pro Gln Leu Gln
    850                 855                 860

ctg cac tgc tca gac gag atc tcc agt cta tgt gct gaa gaa gca gca   2640
Leu His Cys Ser Asp Glu Ile Ser Ser Leu Cys Ala Glu Glu Ala Ala
865                 870                 875                 880

gcc caa gag cag aca ggt cag gtg gag gag tgc ctc aag gtc aac ctg   2688
Ala Gln Glu Gln Thr Gly Gln Val Glu Glu Cys Leu Lys Val Asn Leu
                885                 890                 895

ctc aag atc aaa aca gaa ttg tgt aaa aag gaa gtg cta aac atg ctg   2736
Leu Lys Ile Lys Thr Glu Leu Cys Lys Lys Glu Val Leu Asn Met Leu
            900                 905                 910

aag gaa agc aaa gca gac atc ttt gtt gac ccg gta ctt cat act gct   2784
Lys Glu Ser Lys Ala Asp Ile Phe Val Asp Pro Val Leu His Thr Ala
        915                 920                 925

tgt gcc ctg gac att aaa cac cac tgc gca gcc atc acc cct ggc cgc   2832
Cys Ala Leu Asp Ile Lys His His Cys Ala Ala Ile Thr Pro Gly Arg
    930                 935                 940

ggg cgt caa atg tcc tgt ctc atg gaa gca ctg gag gat aag cgg gtg   2880
Gly Arg Gln Met Ser Cys Leu Met Glu Ala Leu Glu Asp Lys Arg Val
945                 950                 955                 960

agg tta cag ccc gag tgc aaa aag cgc ctc aat gac cgg att gag atg   2928
Arg Leu Gln Pro Glu Cys Lys Lys Arg Leu Asn Asp Arg Ile Glu Met
                965                 970                 975

tgg agt tac gca gca aag gtg gcc cca gca gat ggc ttc tct gat ctt   2976
Trp Ser Tyr Ala Ala Lys Val Ala Pro Ala Asp Gly Phe Ser Asp Leu
            980                 985                 990

gcc atg caa gta atg acg tct cca tct aag aac tac att ctc tct gtg   3024
Ala Met Gln Val Met Thr Ser Pro Ser Lys Asn Tyr Ile Leu Ser Val
        995                 1000                1005

atc agt ggg agc atc tgt ata ttg ttc ctg att ggc ctg atg tgt gga   3072
Ile Ser Gly Ser Ile Cys Ile Leu Phe Leu Ile Gly Leu Met Cys Gly
    1010                1015                1020
```

```
 cgg atc acc aag cga gtg aca cga gag ctc aag gac agg tag          3114
Arg Ile Thr Lys Arg Val Thr Arg Glu Leu Lys Asp Arg  *
1025                1030                1035
```

<210> 6
<211> 1037
<212> PRT
<213> Homo sapiens

<400> 6

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Asp Val Arg Glu Pro Glu Asn Glu Ile Ser Ser Asp Cys Asn His Leu
 1               5                  10                  15
Leu Trp Asn Tyr Lys Leu Asn Leu Thr Thr Asp Pro Lys Phe Glu Ser
            20                  25                  30
Val Ala Arg Glu Val Cys Lys Ser Thr Ile Thr Glu Ile Glu Glu Cys
        35                  40                  45
Ala Asp Glu Pro Val Gly Lys Gly Tyr Met Val Ser Cys Leu Val Asp
    50                  55                  60
His Arg Gly Asn Ile Thr Glu Tyr Gln Cys His Gln Tyr Ile Thr Lys
65                  70                  75                  80
Met Thr Ala Ile Ile Phe Ser Asp Tyr Arg Leu Ile Cys Gly Phe Met
                85                  90                  95
Asp Asp Cys Lys Asn Asp Ile Asn Ile Leu Lys Cys Gly Ser Ile Arg
            100                 105                 110
Leu Gly Glu Lys Asp Ala His Ser Gln Gly Glu Val Val Ser Cys Leu
        115                 120                 125
Glu Lys Gly Leu Val Lys Glu Ala Glu Glu Arg Glu Pro Lys Ile Gln
    130                 135                 140
Val Ser Glu Leu Cys Lys Lys Ala Ile Leu Arg Val Ala Glu Leu Ser
145                 150                 155                 160
Ser Asp Asp Phe His Leu Asp Arg His Leu Tyr Phe Ala Cys Arg Asp
                165                 170                 175
Asp Arg Glu Arg Phe Cys Glu Asn Thr Gln Ala Gly Glu Gly Arg Val
            180                 185                 190
Tyr Lys Cys Leu Phe Asn His Lys Phe Glu Glu Ser Met Ser Glu Lys
        195                 200                 205
Cys Arg Glu Ala Leu Thr Thr Arg Gln Lys Leu Ile Ala Gln Asp Tyr
    210                 215                 220
Lys Val Ser Tyr Ser Leu Ala Lys Ser Cys Lys Ser Asp Leu Lys Lys
225                 230                 235                 240
Tyr Arg Cys Asn Val Glu Asn Leu Pro Arg Ser Arg Glu Ala Arg Leu
                245                 250                 255
Ser Tyr Leu Leu Met Cys Leu Glu Ser Ala Val His Arg Gly Arg Gln
            260                 265                 270
Val Ser Ser Glu Cys Gln Gly Glu Met Leu Asp Tyr Arg Arg Met Leu
        275                 280                 285
Met Glu Asp Phe Ser Leu Ser Pro Glu Ile Ile Leu Ser Cys Arg Gly
    290                 295                 300
Glu Ile Glu His His Cys Ser Gly Leu His Arg Lys Gly Arg Thr Leu
305                 310                 315                 320
His Cys Leu Met Lys Val Val Arg Gly Glu Lys Gly Asn Leu Gly Met
                325                 330                 335
Asn Cys Gln Gln Ala Leu Gln Thr Leu Ile Gln Glu Thr Asp Pro Gly
            340                 345                 350
```

```
Ala Asp Tyr Arg Ile Asp Arg Ala Leu Asn Glu Ala Cys Glu Ser Val
        355                 360                 365
Ile Gln Thr Ala Cys Lys His Ile Arg Ser Gly Asp Pro Met Ile Leu
    370                 375                 380
Ser Cys Leu Met Glu His Leu Tyr Thr Glu Lys Met Val Glu Asp Cys
385                 390                 395                 400
Glu His Arg Leu Leu Glu Leu Gln Tyr Phe Ile Ser Arg Asp Trp Lys
                405                 410                 415
Leu Asp Pro Val Leu Tyr Arg Lys Cys Gln Gly Asp Ala Ser Arg Leu
            420                 425                 430
Cys His Thr His Gly Trp Asn Glu Thr Ser Glu Phe Met Pro Gln Gly
        435                 440                 445
Ala Val Phe Ser Cys Leu Tyr Arg His Ala Tyr Arg Thr Glu Glu Gln
    450                 455                 460
Gly Arg Arg Leu Ser Arg Glu Cys Arg Ala Glu Val Gln Arg Ile Leu
465                 470                 475                 480
His Gln Arg Ala Met Asp Val Lys Leu Asp Pro Ala Leu Gln Asp Lys
                485                 490                 495
Cys Leu Ile Asp Leu Gly Lys Trp Cys Ser Glu Lys Thr Glu Thr Gly
            500                 505                 510
Gln Lys Leu Glu Cys Leu Gln Asp His Leu Asp Asp Leu Val Val Glu
        515                 520                 525
Cys Arg Asp Ile Val Gly Asn Leu Thr Glu Leu Glu Ser Glu Asp Ile
    530                 535                 540
Gln Ile Glu Ala Leu Leu Met Arg Ala Cys Glu Pro Ile Ile Gln Asn
545                 550                 555                 560
Phe Cys His Asp Val Ala Asp Asn Gln Ile Asp Ser Gly Asp Leu Met
                565                 570                 575
Glu Cys Leu Ile Gln Asn Lys His Gln Lys Asp Met Asn Glu Lys Cys
            580                 585                 590
Ala Ile Gly Val Thr His Phe Gln Leu Val Gln Met Lys Asp Phe Arg
        595                 600                 605
Phe Ser Tyr Lys Phe Lys Met Ala Cys Lys Glu Asp Val Leu Lys Leu
    610                 615                 620
Cys Pro Asn Ile Lys Lys Lys Val Asp Val Val Ile Cys Leu Ser Thr
625                 630                 635                 640
Thr Val Arg Asn Asp Thr Leu Gln Glu Ala Lys Glu His Arg Val Ser
                645                 650                 655
Leu Lys Cys Arg Arg Gln Leu Arg Val Glu Glu Leu Glu Met Thr Glu
            660                 665                 670
Asp Ile Arg Leu Glu Pro Asp Leu Tyr Glu Ala Cys Lys Ser Asp Ile
        675                 680                 685
Lys Asn Phe Cys Ser Ala Val Gln Tyr Gly Asn Ala Gln Ile Ile Glu
    690                 695                 700
Cys Leu Lys Glu Asn Lys Lys Gln Leu Ser Thr Arg Cys His Gln Lys
705                 710                 715                 720
Val Phe Lys Leu Gln Glu Thr Glu Met Met Asp Pro Glu Leu Asp Tyr
                725                 730                 735
Thr Leu Met Arg Val Cys Lys Gln Met Ile Lys Lys Phe Cys Pro Glu
            740                 745                 750
Ala Asp Ser Lys Thr Met Leu Gln Cys Leu Lys Gln Asn Lys Asn Ser
        755                 760                 765
Glu Leu Met Asp Pro Lys Cys Lys Gln Met Ile Thr Lys Arg Gln Ile
    770                 775                 780
Thr Gln Asn Thr Asp Tyr Arg Leu Asn Pro Met Leu Arg Lys Ala Cys
785                 790                 795                 800
Lys Ala Asp Ile Pro Lys Phe Cys His Gly Ile Leu Thr Lys Ala Lys
```

```
                805                 810                 815
 Asp Asp Ser Glu Leu Glu Gly Gln Val Ile Ser Cys Leu Lys Leu Arg
            820                 825                 830
Tyr Ala Asp Gln Arg Leu Ser Ser Asp Cys Glu Asp Gln Ile Arg Ile
        835                 840                 845
Ile Ile Gln Glu Ser Ala Leu Asp Tyr Arg Leu Asp Pro Gln Leu Gln
    850                 855                 860
Leu His Cys Ser Asp Glu Ile Ser Ser Leu Cys Ala Glu Glu Ala Ala
865                 870                 875                 880
Ala Gln Glu Gln Thr Gly Gln Val Glu Glu Cys Leu Lys Val Asn Leu
                885                 890                 895
Leu Lys Ile Lys Thr Glu Leu Cys Lys Lys Glu Val Leu Asn Met Leu
            900                 905                 910
Lys Glu Ser Lys Ala Asp Ile Phe Val Asp Pro Val Leu His Thr Ala
        915                 920                 925
Cys Ala Leu Asp Ile Lys His His Cys Ala Ala Ile Thr Pro Gly Arg
    930                 935                 940
Gly Arg Gln Met Ser Cys Leu Met Glu Ala Leu Glu Asp Lys Arg Val
945                 950                 955                 960
Arg Leu Gln Pro Glu Cys Lys Lys Arg Leu Asn Asp Arg Ile Glu Met
                965                 970                 975
Trp Ser Tyr Ala Ala Lys Val Ala Pro Ala Asp Gly Phe Ser Asp Leu
            980                 985                 990
Ala Met Gln Val Met Thr Ser Pro Ser Lys Asn Tyr Ile Leu Ser Val
        995                 1000                1005
Ile Ser Gly Ser Ile Cys Ile Leu Phe Leu Ile Gly Leu Met Cys Gly
    1010                1015                1020
Arg Ile Thr Lys Arg Val Thr Arg Glu Leu Lys Asp Arg
1025                1030                1035


<210> 7
<211> 1177
<212> PRT
<213> Homo sapiens

<400> 7
Met Ala Ala Cys Gly Arg Val Arg Arg Met Phe Arg Leu Ser Ala Ala
 1               5                  10                  15
Leu His Leu Leu Leu Leu Phe Ala Ala Gly Gly Arg Asn Ser Pro Ala
            20                  25                  30
Arg Ala Ser His Ser Gln Gly Gln Gly Pro Gly Ala Asn Phe Val Ser
        35                  40                  45
Phe Val Gly Gln Ala Gly Gly Gly Gly Pro Ala Gly Gln Gln Leu Pro
    50                  55                  60
Gln Leu Pro Gln Ser Ser Gln Leu Gln Gln Gln Gln Gln Gln Gln Gln
65                  70                  75                  80
Gln Gln Gln Gln Pro Gln Pro Pro Gln Pro Pro Phe Pro Ala Gly Gly
                85                  90                  95
Pro Pro Arg Arg Gly Gly Ala Gly Ala Gly Gly Gly Trp Lys Leu Ala
            100                 105                 110
Glu Glu Glu Ser Cys Arg Glu Asp Val Thr Arg Val Cys Pro Lys His
        115                 120                 125
Thr Trp Ser Asn Asn Leu Ala Val Leu Glu Cys Leu Gln Asp Val Arg
    130                 135                 140
Glu Pro Glu Asn Glu Ile Ser Ser Asp Cys Asn His Leu Leu Trp Asn
145                 150                 155                 160
```

```
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Pro Val Gly Lys Gly Tyr Met Val Ser Cys Leu Val Asp His Arg Gly
        195                 200                 205
Asn Ile Thr Glu Tyr Gln Cys His Gln Tyr Ile Thr Lys Met Thr Ala
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225                 230                 235                 240
Lys Asn Asp Ile Asn Ile Leu Lys Cys Gly Ser Ile Arg Leu Gly Glu
                245                 250                 255
Lys Asp Ala His Ser Gln Gly Glu Val Val Ser Cys Leu Glu Lys Gly
            260                 265                 270
Leu Val Lys Glu Ala Glu Glu Arg Glu Pro Lys Ile Gln Val Ser Glu
        275                 280                 285
Leu Cys Lys Lys Ala Ile Leu Arg Val Ala Glu Leu Ser Ser Asp Asp
    290                 295                 300
Phe His Leu Asp Arg His Leu Tyr Phe Ala Cys Arg Asp Asp Arg Glu
305                 310                 315                 320
Arg Phe Cys Glu Asn Thr Gln Ala Cys Glu Gly Arg Val Tyr Lys Cys
                325                 330                 335
Leu Phe Asn His Lys Phe Glu Glu Ser Met Ser Glu Lys Cys Arg Glu
            340                 345                 350
Ala Leu Thr Thr Arg Gln Lys Leu Ile Ala Gln Asp Tyr Lys Val Ser
        355                 360                 365
Tyr Ser Leu Ala Lys Ser Cys Lys Ser Asp Leu Lys Lys Tyr Arg Cys
    370                 375                 380
Asn Val Glu Asn Leu Pro Arg Ser Arg Glu Ala Arg Leu Ser Tyr Leu
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                405                 410                 415
Glu Cys Gln Gly Glu Met Leu Asp Tyr Arg Arg Met Leu Met Glu Asp
            420                 425                 430
Phe Ser Leu Ser Pro Glu Ile Ile Leu Ser Cys Arg Gly Glu Ile Glu
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    450                 455                 460
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705                 710                 715                 720
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Phe Val Gly Gln Ala Gly Gly Gly Gly Pro Ala Gly Gln Gln Leu Pro
    50                  55                  60
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65                  70                  75                  80
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Pro Pro Ala Arg Arg Gly Gly Ala Gly Ala Gly Gly Gly Trp Lys Leu
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Arg Glu Val Cys Lys Ser Thr Ile Thr Glu Ile Lys Glu Cys Ala Asp
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Glu Pro Val Gly Lys Gly Tyr Met Val Ser Cys Leu Val Asp His Arg
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Gly Asn Ile Thr Glu Tyr Gln Cys His Gln Tyr Ile Thr Lys Met Thr
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225                 230                 235                 240
Cys Lys Asn Asp Ile Asn Ile Leu Lys Cys Gly Ser Ile Arg Leu Gly
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Glu Lys Asp Ala His Ser Gln Gly Glu Val Val Ser Cys Leu Glu Lys
            260                 265                 270
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Cys Asn Val Glu Asn Leu Pro Arg Ser Arg Glu Ala Arg Leu Ser Tyr
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Leu Leu Met Cys Leu Glu Ser Ala Val His Arg Gly Arg Gln Val Ser
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Ser Glu Cys Gln Gly Glu Met Leu Asp Tyr Arg Arg Met Leu Met Glu
            420                 425                 430
Asp Phe Ser Leu Ser Pro Glu Ile Ile Leu Ser Cys Arg Gly Glu Ile
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Leu Met Lys Val Val Arg Gly Glu Lys Gly Asn Leu Gly Met Asn Cys
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Met Asp Val Lys Gln Asp His Leu Asp Asp Leu Val Val Glu Cys Arg
            660                 665                 670
Asp Ile Val Gly Asn Leu Thr Glu Leu Glu Ser Glu Asp Ile Gln Ile
        675                 680                 685
Glu Ala Leu Leu Met Arg Ala Cys Glu Pro Ile Ile Gln Asn Phe Cys
    690                 695                 700
His Asp Val Ala Asp Asn Gln Ile Asp Ser Gly Asp Leu Met Glu Cys
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Leu Ile Gln Asn Lys His Gln Lys Asp Met Asn Glu Lys Cys Ala Ile
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    770                 775                 780
Arg Asn Asp Thr Leu Gln Glu Ala Lys Glu His Arg Val Ser Leu Lys
785                 790                 795                 800
Cys Arg Arg Gln Leu Arg Val Glu Glu Leu Glu Met Thr Glu Asp Ile
                805                 810                 815
Arg Leu Glu Pro Asp Leu Tyr Glu Ala Cys Lys Ser Asp Ile Lys Asn
            820                 825                 830
Phe Cys Ser Ala Val Gln Tyr Gly Asn Ala Gln Ile Ile Glu Cys Leu
        835                 840                 845
Lys Glu Asn Lys Lys Gln Leu Ser Thr Arg Cys His Gln Lys Val Phe
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Lys Leu Gln Glu Thr Glu Met Met Asp Pro Glu Leu Asp Tyr Thr Leu
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Met Arg Val Cys Lys Gln Met Ile Lys Arg Phe Cys Pro Glu Ala Asp
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Ser Lys Thr Met Leu Gln Cys Leu Lys Gln Asn Lys Asn Ser Glu Leu
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            980                 985                 990
Gln Glu Ser Ala Leu Asp Tyr Arg Leu Asp Pro Gln Leu Gln Leu His
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Cys Ser Asp Glu Ile Ser Ser Leu Cys Ala Glu Glu Ala Ala Ala Gln
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Leu Asp Ile Lys His His Cys Ala Ala Ile Thr Pro Gly Arg Gly Arg
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Gln Met Ser Cys Leu Met Glu Ala Leu Glu Asp Lys Arg Val Arg Leu
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        35                  40                  45

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    50                  55                  60

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Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr
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Gly Gln Thr Ala Ser Ile Thr Cys Gly Gly Asn Asn Ile Gly Ser Lys
20 25 30

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Ser Val His Trp Tyr His Gln Lys Pro Gly Gln Ala Pro Val Leu Val
35 40 45

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Val Tyr Asp Asp Ser Asp Arg Pro Ser Gly Ile Pro Glu Arg Phe Ser
50 55 60

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Gly Ser Asn Ser Gly Asn Thr Ala Thr Leu Thr Ile Thr Arg Val Glu
65 70 75 80

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Ala Gly Asp Glu Ala Asp Tyr Tyr Cys Gln Val Trp Asp Ser Ser Ser
85 90 95

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Asp Leu Asn Trp Val Phe Gly Gly Arg Thr Gln Ala Asp Arg Pro Thr
100 105 110

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        35                  40                  45
Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu
    50                  55                  60
Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr
65                  70                  75                  80
Cys Ala Arg Ser Thr Thr Arg Ser Tyr Pro Leu Tyr Gly Met Asp Val
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Trp Gly Gln Gly Asn Pro Val Thr
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        35                  40                  45
Val Tyr Asp Asp Ser Asp Arg Pro Ser Gly Ile Pro Glu Arg Phe Ser
    50                  55                  60
Gly Ser Asn Ser Gly Asn Thr Ala Thr Leu Thr Ile Thr Arg Val Glu
65                  70                  75                  80
Ala Gly Asp Glu Ala Asp Tyr Tyr Cys Gln Val Trp Asp Ser Ser Ser
                85                  90                  95
Asp Leu Asn Trp Val Phe Gly Gly Arg Thr Gln Ala Asp Arg Pro Thr
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caagagcaga caggtcaggt gg 22

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
8 December 2005 (08.12.2005)

PCT

(10) International Publication Number
WO 2005/116076 A3

(51) International Patent Classification[7]: C07K 14/705, 16/30

(21) International Application Number:
PCT/IB2005/002480

(22) International Filing Date: 26 January 2005 (26.01.2005)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
10/764,730 26 January 2004 (26.01.2004) US

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(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **VOLLMERS, Heinz, Peter** [DE/DE]; Bonhoefferstrasse 19, 97078 Würzburg (DE). **MÜLLER-HERMELINK, Hans, Konrad** [DE/DE]; Heinrich-Zeuner-Strasse 72, 97082 Würzburg (DE). **HENSEL, Frank** [DE/DE]; Am Exerzierplatz 1, 97070 Würzburg (DE).

(74) Agent: **MBM & CO.**; P.O. Box 809, Station B, Ottawa, Ontario K1P 5P9 (CA).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

(88) Date of publication of the international search report:
6 April 2006

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 2005/116076 A3

(54) Title: NEOPLASM-SPECIFIC POLYPEPTIDES AND THEIR USES

(57) Abstract: The present invention features novel polypeptides and methods of using these polypeptides in the diagnosis, detection, monitoring, and treatment of neaplasms in mammal, e.g., a human.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/IB2005/002480

A. CLASSIFICATION OF SUBJECT MATTER
C07K14/705 C07K16/30

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, EMBASE, BIOSIS, MEDLINE, Sequence Search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 03/011907 A (MUELLER-HERMELINK, HANS, KONRAD; VOLLMERS, HEINZ; HENSEL, FRANK) 13 February 2003 (2003-02-13) the whole document sequences 1-6	1-57
A	VOLLMERS H P ET AL: "HUMAN MONOCLONAL ANTIBODIES FROM STOMACH CARCINOMA PATIENTS REACT WITH HELICOBACTER PYLORI AND STIMULATE STOMACH CANCER CELLS IN VITRO" CANCER, AMERICAN CANCER SOCIETY, PHILADELPHIA, PA, US, vol. 74, no. 5, 1994, pages 1525-1532, XP009015348 ISSN: 0008-543X the whole document	1-57

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
17 November 2005	01/12/2005

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Irion, A

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	HENSEL F ET AL: "Mitogenic autoantibodies in Helicobacter pylori-associated stomach cancerogenesis" INTERNATIONAL JOURNAL OF CANCER, NEW YORK, NY, US, vol. 81, no. 2, 12 April 1999 (1999-04-12), pages 229-235, XP002250371 ISSN: 0020-7136 the whole document figure 3	1-57
A	BRAENDLEIN STEPHANIE ET AL: "Cysteine-rich fibroblast growth factor receptor 1, a new marker for precancerous epithelial lesions defined by the human monoclonal antibody PAM-1." CANCER RESEARCH, vol. 63, no. 9, 1 May 2003 (2003-05-01), pages 2052-2061, XP002354629 ISSN: 0008-5472 the whole document	1-57

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB2005/002480

Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 20-36,48-50,55 are directed to a method of treatment of the human/animal body and/or to a diagnostic method practised on the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/IB2005/002480

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 03011907 A	13-02-2003	CA 2455154 A1	13-02-2003
		CN 1558914 A	29-12-2004
		EP 1423425 A2	02-06-2004
		JP 2005508884 T	07-04-2005
		US 2005032134 A1	10-02-2005